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## **MICRO 2018 Fate and Impact of Microplastics: Knowledge, Actions and Solutions.**

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# MICRO 2018

## FATE AND IMPACT OF MICROPLASTICS: KNOWLEDGE, ACTIONS AND SOLUTIONS.

CONFERENCE PROCEEDINGS



Baztan J., Bergmann M., Carrasco A., Fossi C., Jorgensen B., Miguelez A., Pahl S., Thompson R.C., Vanderlinden J-P.

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**November 19th: 8h30 Registration desk in el Cabildo de Lanzarote.**

**November 19th: 9h30 Official Welcome.**

**Posters displayed from 19 to 23 November in the Arrecife Gran Hotel.**

**Oral presentations, length 15' max.**

## **Keynotes and public discussions:**

### **18h-19h15, Tuesday Nov. 20<sup>th</sup>**

Science for Policy:

Marine Strategy Framework Directive - Technical Group Marine Litter Position Paper, Georg Hanke, JRC.

Panel Discussion :Andy Booth, Francesco Regoli, Annika Jahnke, Gunnar Gerdts and TG Marine Litter Representatives.

### **19h30-20h30, Wednesday Nov. 21<sup>st</sup>**

The Megafauna and microplastics tale: Evidence and future development, 25' talk by Maria Cristina Fossi.

Plastic Is Forever, a 19' film by Dylan D'haeze.

Public discussion.

### **19h-20h, Thursday Nov. 22<sup>nd</sup>**

Marine Litter: Are there solutions to this global environmental problem?, talk by Richard Thompson.

Public discussion.

Concert.

**November 23<sup>rd</sup> 17h, Closing Event...**  
**...see you at MICRO 2020 !**

# **Oral presentations, by day and panel**



## November 19th

### November 19th – Plenary 1: 10h - 12h (El Cabildo)

#### Degradation of Polyolefin Plastics Under Marine Exposure Conditions

Anthony Andrady \* 1, Kara Lavender-Law , Giora Proskurowski , Jessica Donohue

*1 University of North Carolina at Wilmington – College Street, Wilmington, NC 28403, United States*

Solar UV-B radiation and temperature are well known to be the key agents of degradation of plastics debris in the marine environment. In this study a laboratory accelerated weathering experiment was carried out on polyethylene (LDPE and HDPE), polypropylene (PP) and a photodegradable ethylene copolymer commonly used in degradable packaging applications. Exposures were carried out with samples in air and floating in sea water and the progress of degradation was assessed by changes in mechanical integrity, surface cracking and spectral changes indicative of oxidation of the polymers. The data shows that loss in mechanical integrity of the plastics exposed in seawater cannot be explained solely by classical mechanisms of oxidative scission of polymer chains. potential changes in crystallinity afforded by the swelling of plastics in water. The efficacy of the photodegradable technology based on ethylene-carbon monoxide copolymers for plastics exposed while floating in seawater will be demonstrated. The implications of these results, especially those on the photodegradable copolymer, on the fragmentation of litter into mesoscale and nanoscale plastics will also be discussed.

Keywords: Degradation, Seawater, oxidation

\*Speaker

#### Clean Coast: project for the study and management of oceanic waste in the Peninsula de Guanahacabibes Biosphere Reserve, Cuba.

Lázaro Márquez Govea \* 1, Odismarlyn Blanco Blanco 2

*1 Peninsula de Guanahacabibes Biosphere Reserve – Cuba 2 Pinar del Rio University – Cuba*

The Peninsula de Guanahacabibes Biosphere Reserve is the only Antillean island territory with coasts in the Caribbean Sea and in the Gulf of Mexico. This unique geographic location conditions the richness and diversity of its biota, but also causes it to be bordered by a complex system of intense marine currents that annually transport tons of waste, mainly plastics, to its coasts. This phenomenon causes serious environmental damage with incalculable effects on biodiversity and affects the tourist activities in the area by reducing the attractiveness of beaches and coastal areas. The Clean Coast project is an initiative based on citizen participation that aims to determine the volume of oceanic waste that is arriving at the biosphere reserve, clean the coasts and use the waste. For the evaluation of the waste volume, 5 stations were established in the south coast, in which plots of variable area were defined according to the site. The cleaning of beaches is done through manual collection with the support of university and pre-university students and residents of local communities. The collected waste is classified and weighed by types and used for different uses. The project includes an educational component focused on raising awareness among citizens and training volunteers through conferences, workshops and practical training. As a result, large sectors of the coastal zone have been cleaned, a considerable volume of waste has been collected, mainly plastics, which are used for recycling and in the manufacture of utilitarian objects. The environmental awareness of young people and of the population and local authorities has been increased.

Keywords: oceanic waste, plastics, biodiversity, local communities

\*Speaker

## Do antifouling paint particles pose a risk to sediment-dwelling biota?

Matthew Cole \*† 1, Christina Muller-Karanassos 1,2, William Arundel 1,2, Thomas Vance 1, Andrew Turner

1 Plymouth Marine Laboratory – United Kingdom 2 Plymouth University – United Kingdom

Exposure to microplastics has been shown to incite sub-lethal health effects and affect feeding, growth and reproduction in marine invertebrates. Antifouling paints are widely applied to ships, pontoons and buoys to prevent the growth of fouling organisms; these biocidal paints contain high concentrations of metals (e.g. copper and zinc) that are toxic to microalgae and microorganisms, or fluorocarbons that prevent the adherence of fouling organisms. Mechanical damage, weathering and maintenance can all contribute to the environmental release and accumulation of ‘antifouling paint particles’ (APPs) in estuarine sediments. Owing to their high metal content, we hypothesise that

APPs will pose a significant health risk to marine life. In this study, we investigated the risk microplastics and APPs present to the health of benthic invertebrates. Toxicity studies were conducted using the ragworm *Hediste diversicolor* and the common cockle *Cerastoderma edule* exposed to clean (i.e. controls) and APP-contaminated sediments for up to 28 days. Toxicity was measured through the use of sub-lethal (e.g. metallothionein upregulation), behavioural and apical (e.g. growth, survival) endpoints. Our studies elucidate the risks microplastics containing high concentrations of metals pose to the health of estuarine and coastal biota.

Keywords: paints, microplastics, ecotoxicology

\*Speaker †Corresponding author: mcol@pml.ac.uk

## Global Plastic Waste Fluxes from Coastal and Riverine Sources Doubled between 1990 and 2015

Kevin Lang \*† 1,2, Meike Vogt 2, Charlotte Laufkötter 1,3

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Marine plastic pollution is an increasing global concern, as plastic particles are found everywhere in the ocean and pose various threats to marine life. First estimates quantify the contemporary coastal or riverine plastic input at the country-level, but little is known about the spatio-temporal distribution of its sources and sinks at the global scale. We use machine-learning algorithms to estimate the global plastic input from coastal sources and riverine input for the years 1990-2015 on a 0.1x0.1° grid. Using neural-network and random-forest models, we predict the waste generation per country from socio-economic factors such as population density or GDP. We then apply population size, waste management and composition, as well as distance-based probabilities of land and river transport to derive the plastic flux to the ocean (figure 1). Additionally, the effect of several waste management practise scenarios on plastic fluxes and the sensitivity of the waste production models to parameter choices are assessed. Using a Lagrangian particle-simulator driven by geostrophic current data we determine the fate of the floating marine plastic debris for each scenario.

In line with previous findings, predicted global plastic input increased from 3.5Mt in 1990 to 7.9Mt in 2015 with 88% and 12% entering via coasts and rivers respectively in 2015. While the five countries with the highest modelled plastic input accounted for 55% of the global input in 2015, their fraction was only 43% in 1990 and the top eight countries account for 54%. Our models also

suggest an increasing impact in several African and East-Asian countries in the time period observed while European and Northern-American countries generally show a decrease. Our results show the temporal and spatial development of marine plastic debris generation and the influence of the socio-economic factors. This helps to assess where possible measures to reduce plastic pollution will be most effective.

Keywords: oceanic plastic input, modelling

\*Speaker †Corresponding author: langk@student.ethz.ch

## Use of the risk assessment paradigm to organise and prioritize microplastic research needs

Albert Koelmans \* 1, Ellen Besseling , Noël Diepens 2, Merel Kooi , Svenja Mintenig 3,4, Nur Hazimah Mohamed Nor 5, Paula Redondo Hasselerharm 6

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Researcher and media alarms have caused plastic debris to be perceived as a major threat to humans and animals. However, although the waste of plastic in the environment is clearly undesirable for aesthetic and economic reasons, the actual environmental risks of different plastics and their associated chemicals remain largely unknown. Understanding the behaviour and ecological risks of plastic debris in the environment is a crucial prerequisite for finding feasible solutions. We analyse the problem using a systematic risk assessment approach. We show how a systematic assessment of adverse outcome pathways based on ecologically relevant metrics for exposure and effect can bring risk assessment within reach. This implies assessing and predicting environmental concentrations (PECs) and ecologically relevant effect thresholds (LOECs), in order to be able to quantitatively characterise risk. To increase ecological relevance, effect assessment includes the use of Species Sensitivity distributions (SSD). Mechanistic modelling approaches are used to (a) allow interpolation between experimental and field data that otherwise remain fragmentary and patchy, and (b) extrapolate to domains where measurement is not yet possible, like the future or inaccessible parts of the oceans. Model studies are complemented with experimental and field data to verify trends and mechanisms that emerge from first principles. In the presentation a systems analytical view on behaviour and effects of plastic debris and a provisional yet full ecological risk assessment

will be provided. Results of such an assessment will help to respond to the current public worry in a balanced way and allow policy makers to take measures for scientifically sound reasons.

Keywords: exposure, effects, environmental modeling, risk assessment

\*Speaker

## Understanding the effects of the plastic additive Bisphenol A (BPA) on estuarine food webs

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Bisphenol A (BPA) is a synthetic phenol extensively used in food packaging materials, dental sealants, medical devices and other human-consumption products. Consequently, exposure to BPA is ubiquitous via ingestion, inhalation, and dermal contact. On the last decades, numerous studies have linked BPA to diseases such as cancer, diabetes, obesity, and various disorders in the reproductive, neuronal, immune, and cardiovascular systems. Nowadays manufacturers are switching away from BPA-based consumer plastics and using various "BPA-free" alternatives (BPA substitutes, such as Bisphenol S- BPS), yet bisphenols are found at relatively high concentrations in rivers, lakes and estuaries worldwide. On the other hand, it is known that BPA properties (e.g. leaching) can change with temperature. Evidence exists for transfer and uptake of plastic additives by marine organisms, where contamination may occur via natural pathways (i.e. waterborne or foodborne exposure) or via ingestion of plastic debris including MP. Here, we use a calibrated ecosystem-model from a temperate estuary to understand and project potential effects of BPA on estuarine food webs, particularly, under scenarios of climate change. Concentrations of BPA measured in estuaries are used to force the model, as well as temperature oscillations according to IPCC scenarios for temperate latitudes. We discuss the effects of BPA at the trophic-group level and at the whole-system level and analyse potential interactions with temperature rise. Preliminary simulations highlight the need for further data on ecotoxicological parameters of the various trophic levels encompassing estuarine food webs.

Keywords: BPA, Plastic additive, estuary, ecosystem, model

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## November 19th – Plenary 2: 14h30 - 17h (Arrecife Gran Hotel)

### Are there microplastics in drinking water? – A study on microplastics occurrence at different water treatment plants, Central Europe region.

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The study examined the occurrence of microplastics (MPs) in raw and treated drinking water obtained from different water treatment plants (WTPs) located within urban areas of the Czech Republic, Central Europe. Three WTPs (WTP1-3) supplied by diverse kinds of water bodies and applying distinct treatment technologies were selected. A substantial content of MPs was determined in all samples, i.e.  $1473 \pm 34$ ,  $1812 \pm 35$  and  $3605 \pm 497$  particles/L in raw water of WTP1, WTP2 and WTP3, respectively, and  $443 \pm 10$ ,  $338 \pm 76$  and  $628 \pm 28$  particles/L, resp., in treated water. Although the number of MPs was always significantly lower (by 83% on average) in treated than in raw water, considerable amounts of microplastics remained unremoved. Besides the quantification of MPs, they were also characterized in terms of their size, shape and material composition. Microplastics down to 1  $\mu\text{m}$  were determined and divided into five size classes (1-5  $\mu\text{m}$ ; 5-10  $\mu\text{m}$ ; 10-50  $\mu\text{m}$ ; 50-100  $\mu\text{m}$ ; > 100  $\mu\text{m}$ ), as shown in Figure 1. The MPs belonging to the first category (1-5  $\mu\text{m}$ ) prevailed, comprising approximately 40-60% in raw water and 25-60% in treated water, and in general, the vast majority was smaller than 10  $\mu\text{m}$  (up to 95%). Concerning the shape of microplastics, fragments were the most plentiful in WTP1 and WTP2 raw water, while fragments together with fibres predominated in the case of WTP3. Similar pattern was observed for treated water samples, except for an increase in fibres proportion at WTP3, which might indicate their less efficient removability by the employed technology. Further, 12 different materials forming the MPs were identified, while PET (polyethylene terephthalate), PP (polypropylene) and PE (polyethylene) particles were the most abundant. These results contribute to fill the knowledge gap regarding the emerging microplastic pollution related to drinking water supply.

Keywords: Central Europe, drinking water sources, microplastics, plastic contamination, water

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# Accumulation of plastic debris and associated contaminants in aquatic food webs

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Hydrophobic Organic Contaminants (HOCs) are known to biomagnify in marine food webs. However, the extent to which microplastics accumulate in food webs and how this influences the uptake of HOCs remains unclear. We present a generic theoretical model (MICROWEB) that simulates the transfer of microplastics and hydrophobic organic chemicals (HOC) in food webs. We implemented the model for an Arctic case comprised of nine species including Atlantic cod, with polar bear as top predator. We used the model to examine the effect of plastic ingestion on trophic transfer of microplastics and persistent HOCs (PCBs) and metabolizable HOCs (PAHs), spanning a wide range of hydrophobicities. In a scenario where HOCs in plastic and water are at equilibrium, PCBs biomagnify less when more microplastic is ingested, because PCBs biomagnify less well from ingested plastic than from regular food. In contrast, PAH biomagnify more when more microplastic is ingested, because plastic reduces the fraction of PAH available for metabolism. We also explore non-equilibrium scenarios representative of additives that are leaching out, as well as sorbing HOCs, quantitatively showing how the above trends are strengthened and weakened, respectively. The observed patterns were not very sensitive to modifications in the structure of the food web. The model can be used as a tool to assess prospective risks of exposure to microplastics and complex HOC mixtures for any food web, including those with relevance for human health.

Keywords: marine plastic debris, contaminants, Atlantic cod, food web modelling, trophic transfer, bioaccumulation, human health

\*Speaker

# What about us? Effects of nano- and microplastics on human immune cells

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Recent research has mostly investigated nano- and microplastic toxicity in aquatic organism while data on human health is scarce. Prior research on plastic implant abrasion indicates an involvement of plastic particles in inflammatory responses, however, extensive experimental data is lacking. Thus, the aim of our study is to assess the effects of nano-/microplastic spheres (polystyrene (PS); 50, 100, 300 nm) and fragments (PS, polymethylmethacrylate (PMMA), polyvinyl chloride (PVC); 50-2000 nm) on the pro- and anti-inflammatory cytokine signaling of human immune cells (monocytes (MO), dendritic cells (DC)). MO (n=6) from blood donor samples and MO-derived DC (n=5) were exposed to these particles (100 particles per cell) over 16 hours. Additionally, we included an unexposed control, aqueous leachates of the particles and a detergent control (0.01% Tween20 for PS fragments) as treatments in the test design. Cytokine expression in response to the particle exposure was measured both in absence and presence of lipopolysaccharides (LPS), which simulate a bacterial infection (MO: 1 µg mL<sup>-1</sup>, DC: 50 ng mL<sup>-1</sup>). Post-exposure concentrations of the pro-inflammatory cytokines IL-6 and TNF-α and the anti-inflammatory cytokine IL-10 in the supernatant were determined using ELISA. In MO and DC treatments without LPS stimulation, PS, PMMA (only DC) and PVC fragments enhanced pro-inflammatory cytokine secretion (Fig.1). The anti-inflammatory response in MO increased in PMMA and PVC fragments treatments and decreased for the PS treatment. The leachates of the fragments induced cytokine expression in MO, however to a lower extent compared to the particle treatments. With LPS stimulation, exposure to PS and their leachate increased cytokine expression. In contrast, nano-sized PS spheres partially reduced cytokine expression both in presence and absence of LPS. These results indicate that plastic particles may have differential effects on the human immune system provided that the particles reach the human circulatory system in relevant quantities.

Keywords: human, monocytes, dendritic cells, nanoplastics, microplastics, inflammation, cytokines

\*Speaker

## Occurrence of Microplastics in Commercial Seafood from Korea and Human Exposure

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The amount of plastics produced worldwide is expected to increase to 966 million tons in 2050. It is clear that this increase in plastic production will lead to an increase in the extent of microplastic pollution in the environment. Microplastics ultimately enter into the marine environment, and they are often mistaken for food by marine organisms. Despite concern to human exposure via seafood consumption, there are limited researches that extensively investigated the most consumed seafood so far. Particularly, Asian coastal region is known as a global hot spot of plastic emission and pollution (Lebreton et al., 2017). In this study, we collected various taxonomic groups of seafood which are popularly consumed in Korea to quantitatively assess microplastic exposure of Koreans via seafood consumption. For that, nine species of seafood including shellfish (n=4), cephalopod (n=2), crustacean (n=2), and fish (n=1) were collected from three major seafood markets in Korea in February 2018. Analysis of all samples were trip-licates (i.e., total number of samples = 81; 3 markets × 9 species × 3 replicate). For qualitative and quantitative determination of microplastics, we developed a methodology to detect the microplastics remaining in seafood. Organic matter was decomposed overnight at 40 and 120 rpm in a shaking incubator using 10% KOH, and filtered using a 20µm stainless mesh filter. Then, the mesh filter was treated with 17.5% H<sub>2</sub>O<sub>2</sub> and sonicated to remove residual organic matter. Finally, the filtered sample was identified using µ-FTIR (Bruker LUMOS). This procedure generated average recovery of 97.8 ± 2.9%. In blank samples, cotton fibers were detected but no microplastics were found. In this meeting, we will present the analysis results of microplastics in Korean seafood and human exposure assessment. Our result will be also compared with those from other regions.

Keywords: marine organism, seafood, human exposure

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## Investigating the presence of microplastic in Danish drinking water

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Microplastic is a pervasive pollutant found throughout the environment. However, the extent to which humans are exposed to this contaminant is not well researched. Recently studies have indicated that microplastic is also present in tap water, bottled water and food destined for human consumption. Here, we investigate the presence of microplastic in drinking water from 17 sites

around Denmark. 50 L of drinking water was sampled per site directly from taps through 10 µm steel filters in a closed metal filter system to prevent contamination. These filters were then examined visually and representative particles with sizes > 100 µm were identified as either potential microplastic or of uncertain origin and were picked and placed on ZnSe discs. These potential microplastics were then identified using advanced µFTIR utilizing focal plane array (128 x 128) mapping with a pixel resolution of 5.5 µm. 44% of the visually picked particles were validated with µFTIR. These particles consisted of cellulose (76%) and microplastic (3%) such as PET, PP, PS and PMMA. The remaining particles could either not be identified using reference libraries and characterised as "unknown" (7%) because of poor match with spectra in reference libraries or due to poor spectra quality (10%) or were protein-like (4%). Three samples were also passed through Anodisc membrane filters (0.2 µm) suitable for µFT-IR analysis to detect microplastic < 100 µm. This process meant that no visual picking of microplastic was needed before analysis removing any potential for visual bias. 10% (49 mm<sup>2</sup>) of the Anodisc area were mapped with µFTIR imaging with single microplastic fragments of PET, PP as well ABS identified. This study shows that microplastic is present in Danish drinking water, although in significantly lower levels than the studies that were reported for other countries in 2017 that were highly publicised by the media.

*Keywords: Drinking Water, µFTIR, Focal Plane Array*

*\*Corresponding author: jak@bios.au.dk †Speaker*

## Development of a jet pump based microplastic sampling system for freshwaters

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Freshwater studies have mainly been adopting sampling methods from marine research, thus the most commonly used tool is the plankton or manta net, usually with a mesh size of 300 µm or 333 µm. There are certain conditions when application of these sampling systems is complicated on smaller freshwater bodies. Also, the filtered water volume is not obviously defined (due to clogging) even if a flow meter is used. To avoid these difficulties, another sampling approach is needed. The technique using a pump and a set of filters is more common during wastewater sampling than in lakes or rivers. Until now, only a few studies have presented sampling systems that were based on a submersible or a jet pump, including stainless steel filters.

We developed a mobile sampling system that has many benefits when it is applied in inland freshwaters. The size of the complete apparatus enables sampling from a smaller boat or from the shore as well. A jet pump is operated by an aggregator. A PVC hose with a brass foot valve including a 2 mm mesh size strainer is put right under the water surface and is connected to the pump. Water is filtered through a set of 10” stainless steel filter cartridges (variable mesh size) in stainless steel housing. Water quantity is measured by a flowmeter precisely. To prevent clogging of the fine mesh size filter (60  $\mu\text{m}$ ), we coupled two cartridges and put in a 300  $\mu\text{m}$  pre-filter. This system enables sampling of more than 2.000 L water even if the Secchi depth is no more than 10 cm (e.g. due to flood or plankton). Project no. KFI 16-1-2017-0477 has been implemented with the support provided by the National Research, Development and Innovation Fund of Hungary, financed under the "Vállalati KFI 16" funding scheme.

Keywords: microplastic, freshwater, jet pump, sampling

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## High Concentrations of Microplastics in the Bed Sediments of the Rhine River

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Knowledge about the fate of microplastics in rivers is still scarce. Here, we analysed 25 sediment samples at water depths of 5–7.5 m from the bed of the Rhine River at the German locations of Koblenz (n = 10) and Rees (n = 15). The samples were taken from the riverbed with a diving bell (n = 19, undisturbed, sediment depth: 7 cm) and with a bucket chain of a shipping channel maintenance vessel (n = 6, disturbed, depth: 0.5–1.1 m). Density separation on pooled samples (n = 3) was performed using an adapted ZnCl<sub>2</sub> protocol, prior to purification with Fenton's reagent and chemical analysis using ATR-FTIR and FTIR imaging. Our study indicates high concentrations of microplastic particles (< 0.5 mm) in riverbed sediments (264.63– 11,073.71 kg<sup>-1</sup>). Microplastic particles smaller than 75  $\mu\text{m}$  accounted for an average of  $96 \pm 6\%$  (SD). Eighteen different types of synthetic polymers were detected in the samples. Most frequently acrylates/polyurethane (PUR), polystyrene (PS) and polypropylene (PP) were found. Non-expanded PS and high-density PUR and

may weigh up to 1.05 g cm<sup>-3</sup> and 1.42 g cm<sup>-3</sup> respectively, and thus could be expected to sink in freshwater. However, the high PP (0.85 g cm<sup>-3</sup>) findings were unexpected in the sediments of a turbulent waterbody. Data will be discussed in the light of the microplastic data from surface water and riverine sites. The presented data suggests that riverbed sediments may act as an important sink for riverine microplastics.

Keywords: microplastic\*, sediment, river

\*Speaker

## A methodology to characterize macroplastic dynamics in rivers

Tim Van Emmerik \*† 1, Thuy-Chung Kieu-Le 2, Michelle Loozen 3, Kees Van Oeveren 3, Emilie Strady 4, Johnny Gasperi 5, Laurent Lebreton 3, Boyan Slat 3, Bruno Tassin 5

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Land-based macroplastic is considered one of the major sources of marine floating plastic. However, estimations of plastic emission from rivers into the oceans are scarce and uncertain, mainly due to a severe lack of consistent observations. Especially detailed information on spatiotemporal variation in plastic quantities and composition is crucial for assessing plastic fluxes, and for potential mediation and recycling efforts. We present a new methodology to characterize macroplastics in rivers. Furthermore, we discuss results from a first application in the Saigon river, Vietnam. During a two-week period, hourly cross-sectional profiles of plastic flux were made across the river width. Simultaneously, sub-hourly samples were taken to determine the weight, size and composition of riverine macroplastics. Finally, extrapolation of the observations based on available hydrological data yielded estimates of daily, monthly and annual macroplastic emission into the ocean. Results suggest plastic emissions by the Saigon river are up to 4 times as large as previously estimated.

Keywords: rivers, hydrology, method, measurements, plastic pollution, macroplastic, Vietnam

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**November 20th**

**November 20th – Panel 5.1: 8h30 - 10h (AGH),  
Panel chaired by Mateo Cordier.**

## **OC-TECH: PROTECTING THE MARINE ENVIRONMENT THROUGH INNOVATION**

Santiago Miranda \*

OCEAN CLEANER TECHNOLOGY, SL (OCT) has decided to focus on the industrial manufacturing of the OC-TECH innovative vessel, which is protected by patents in Europe and in other countries of the world. Spills' accidents on the sea occur at random and it is therefore impossible to predict the nature, place, time and magnitude of an accident. Pollution control equipment must be permanently available, while being put to profitable use in the periods between clean-up operations.

Two-fold benefits are presented by OC-TECH collecting a wide range of waste materials from marine environments (cleaning) and offering a versatile boat capable to perform the full range of port, oil industry or ocean-going tasks (workboat). This Innovative project has been selected and financed by the European Commission through Horizon 2020 Programme Phases 1 and 2.

The OC-TECH project aims at offering an innovative polyvalent and versatile vessel that is able to recover all types of floating and liquid waste as hydrocarbon spills, to deal with micro algal blooms or to collect flotsam as floating containers or microplastics.

Our boat represents a suitable and highly efficient tool for smart coastal and hydrological cities.

We highlight some of our innovations:

- Self-regulating collection system: There is a multifunctional recovery system inside the tunnel, capable of collecting liquids spills or floating waste. All the operative decantation and filtering activities, except bagging, are performed under the deck of the boat. - On-Board polyethylene bagging system: Spill and other contaminants can be contained safely during OC-TECH operation in large floating bags, which once filled are sealed, are marked with a buoy and towed away by an unspecialized auxiliary vessel for removal and treatment. For small spills and quantities of solids, OC-TECH'S on-board tanks will be used for storage and later, safely unloaded in port.

Keywords: OC, TECH innovative vessel

# The Plastics Manufacturers efforts to prevent marine litter

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Plastics have become indispensable to our lives and are too valuable to be lost in our environment and the oceans. However, plastic debris form a major component of marine litter, negatively affecting aquatic life, leading to socio-economic costs and representing waste of valuable resources. Plastic products need to be responsibly managed throughout their life cycle, so that they do not leak into the environment. In 2015, PlasticsEurope joined the Operation Clean Sweep (OCS) programme, a voluntary industry initiative on plastic pellets loss. Plastic pellets are usually produced in the microsize range to facilitate transport and conversion into products. OCS aims at improving awareness, promoting best practices and providing guidance and tools to support the plastic value chain in the implementation of prevention measures.

PlasticsEurope has committed to:

- To have 100% of its member companies sign the OCS pledge to which OCS is applicable by the end of 2018.
- Develop by 2018 a transparent, harmonised monitoring scheme for the collection of relevant and comparable information from all signed members to measure progress. Reports on progress will be available to all key stakeholders on a yearly basis.
- In 2019 PlasticsEurope will explore the feasibility to develop a common assessment tool with its members.
- To engage with at least one other major European port by the end of 2018. By 2030 we aim at securing that all major industrial plastic pellet handling ports in the EU have taken measures to implement OCS.
- To expand the work of the platforms with the supply chain and relevant stakeholders to accelerate the implementation of OCS within the plastics industry.

At the conference we will present the latest efforts and achievements to prevent plastics pellet loss.

\*Speaker

Keywords: plastics pellet, operation clean sweep, microsize, best practice sharing

# Acute need of Legal Framework for management of marine debris and Microplastics in India

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India is the second most populous Nation of the world. Very high density of population puts a big pressure over country's natural resources. Everyday India produces around 175,000 metric tons of solid waste, 65% of it gets collected, 15% of the total waste gets treated, 50% of the total waste is dumped into the low-lying areas and in landfill sites and 35% remains scattered in the environment. Plastics comprises 7 to 10% of total solid waste by volume, with increasing income of average Indian, plastics will be 25% of total solid waste by 2030. The present annual consumption of plastic in India is around 13.4 million metric tons which will grow to 22 million metric tons by 2020. The average per capita plastic consumption in India is 11 kg per annum against world average of 28 kg. The basic studies about concentration and abundance of micro-plastics in Indian rivers and oceans are not available. However, based on random studies, it is evident that microplastic contamination is increasing drastically in Indian marine environment. In absence of scientific treatment of solid waste and lackadaisical approach in enforcement of legal provisions about solid waste management in India, it pauses a big challenge for future. In this paper it is projected that potential concentration of micro-plastics in Indian marine environment will be significantly higher than the global average and if it is not addressed in scientific and timely manner, it will be one of the major roadblock on sustainable development of not only of India but also of the whole humanity. India has articulated Environment Protection Act (EPA) 1986. However, in present legal framework Microplastics or marine debris is not defined or even mentioned. Therefore, it is high time that India should have inclusion of microplastic management in the existing legal framework.

Keywords: Solid Waste, Legislative Framework, Micro plastics, Marine Debris

\*Speaker

## Development of a novel technological approach for the reduction of microplastic pollution in seawater desalination plants and for sea salt extraction.

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An increasingly and widespread problem is the introduction of plastics into the water cycle. The poor degradability leads to the plastic waste remaining in the water and over time it fragments into smaller and smaller plastic particles, so called microplastics [1]. In case of seawater use (e.g. desalination or sea salt extraction) micro particles represent a significant potential for blocking the microfiltration membranes (pore size > 100 nm) in the pretreatment and reverse osmosis (RO) membranes (pore size > 5 nm).

Our innovative approach for the removal of microplastics from seawater for industrial use combines a chemically induced agglomeration and a new technological implementation step. The particular challenge in removing the microplastics is not only their small size but also their inert properties against most of the physical and chemical additives for flocculation [2]. The concept is based on the application of organosilanes, which can attach to microplastic particles by forming an inclusion compound with simultaneous particle growth (Figure) [3].

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Keywords: microplastics, removal, seawater, seawater desalination, sea salt extraction, organosilanes

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## Treatment stage effect on microplastic abundance and fate during passage through a tertiary wastewater treatment plant

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Microplastics (MPs; < 0.5 mm) are classed contaminants of emerging concern but currently are not regulated by water quality standards. Quantitative and qualitative data of these materials in aquatic systems remains limited, particularly for freshwaters and wastewater treatment systems that are important transport vectors of land-based contaminants to oceans. This study was conducted in a



tertiary wastewater treatment plant in the UK (Glasgow, Scotland) to characterise and quantify MPs in the system and assess the stepwise effect of treatment stage in their abundance and fate before final effluent discharge. The MPs were present during all sampling campaigns and in all treatment stages, ranging from 1-23 items per L, but their prevalence exhibited a downwards trend from inflow to outflow. Fibres comprised 66.59% of all items, followed by films (18.27%) and fragments (14.60%), while abundances of primary MPs were minimal. These findings suggest that MPs pollution is continuous, especially from secondary sources, and while current treatment processes efficiently capture MPs, some particles may still be discharged. However, blank and recovery tests using MPs-spiked samples showed evidence of background contamination of fibres and production of fragments during the extraction protocol that if not considered could lead to over-estimation of MPs abundance. Wastewater treatment systems are expected to play an increasingly important role in regulating MPs pollution, thus this study contributes to understanding of wastewater effluent as a source of MPs and to identify where controls should be implemented.

Keywords: microplastics, freshwater, wastewater, microfibres

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## Understanding the retention of nanoplastics at rock and mineral surfaces

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In India, according to 2015 statistics announced in the parliament, 60 major cities are churning out 3500 tonnes of plastic waste every day and contributes a major share to the world's overall generation. The rapid development of technological innovation has opened the door for utilization of vast variety of plastics in many industrial sectors and majority of these plastics are disposable. Further degradation of microplastics by several pathways might result in the formation of nanoplastics (< 1000 nm). Nanoplastics, due to their high surface-to-volume ratio, may demonstrate distinctive chemical, physical, and biological properties compared to their bulk material properties. The nanoplastics because of their size poses two potential problems 1) can be easily ingested into marine organisms which emphasizes the high risk of disruption to marine food-web 2) high efficiency to sorb contaminants and transport to longer distances to contaminate the nearby aquifers. Hence, the interaction of nanoplastics with the rock and mineral surfaces is crucial to assess their transport in the environment. Underlining the above points, we have recently

published several publications emphasizing the importance of size of nanoplastics on their mobility, roughness of the interacting media, hydrodynamics and charge heterogeneity of mineral substrates and clay particles, including the impact of metal ion concentration on nanoparticle retention under electrostatically unfavorable conditions. Here the deposition of nanoplastics on varying mineral substrates such as calcite, micrite and granodiorite was presented. The results predicted for nanoplastic retention at rock and mineral surfaces 1) roughness of mineral surface plays an important role under unfavourable conditions 2) there exists a critical velocity that is a function of ratio of particle size to roughness of substrate beyond which particle is removed from the mineral surface 3) an increase in contaminant concentration (metal) will overwrite the surface heterogeneities of the mineral for its retention.

Keywords: Nanoplastics, deposition, transport, rock and minerals, roughness

\*Speaker

## **November 20th – Panel 5.2: 8h30 - 10h (EC), Panel chaired by Pennie Lindeque.**

### **From the sewer to the sea - microplastic transport and fate in freshwater systems**

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Microplastics have been detected in rivers and streams around the world. Although these measurements shed light on the occurrence of microplastics in freshwater systems, they are fragmentary in both space and time. Moreover, the measurements cannot reveal all sources or transport characteristics, and therefore hamper the development of exposure assessments. Models can help to integrate these fragmentary data into more comprehensive exposure overviews needed for risk assessments. The models currently available are however focussing on small (sub) catchments or are on a very coarse scale. In this study we modelled the transport and fate of different microplastics in the Netherlands. Based on the WFD Explorer 2.2., a national-scale mass balance model, the Dutch water network was divided into almost 19000 surface water units. We identified and quantified different point sources including over 300 waste water treatment plants and 9 cross-boundary flows, but also took into account diffuse surface runoff of tire wear particles. Emission input data were based on existing literature. We modelled the transport of different polymers, both floating and settling, and analysed the effect of particle size on the fate of microplastics. Also, we calculated the export of microplastics to the North Sea and Lake IJssel. We compared our model results with measurements from both Dutch and non-Dutch rivers. Our model provides the first comprehensive exposure assessment on a national scale, and will form the basis for a prospective risk assessment, since modelled concentrations can be compared with effect thresholds for different aquatic organisms. Also, management measures can be included in the model, to illustrate how these measures would affect microplastic concentrations in freshwater systems.

Keywords: rivers, microplastics, modelling. \*Speaker

# Microplastics going Dutch: A systems approach to understand microplastics measured in riverine surface waters and sediments

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Our understanding of microplastic pollution in freshwaters is lagging behind. Although receiving more attention, sources, pathways and fate of microplastics in rivers need to be investigated more. In this study microplastics were examined in the surface water and sediment of two river systems in the Netherlands. The Dommel is an intermediate river with clear catchment boundaries, its water originates mostly from discharges of various waste water treatment plants (WWTPs). The second river, the Meuse, has a much higher discharge and is mostly rain fed. Samples were taken over the lengths of both rivers. The sampling locations were chosen to, firstly, quantify microplastics in the effluents of WWTP, as well as up- and downstream of the discharging points to assess the relative contribution of WWTPs to the total riverine plastic concentration. Secondly, microplastics were determined in surface waters and sediments in sedimentation and resuspension areas to better understand the effects of river dynamics. This was repeated in Meuse-related drinking water basins. Thirdly, seasonal and daily variations of microplastic concentrations in surface water and WWTP effluents were assessed. Sampling the effluent was repeated under normal weather conditions and during a strong rain event with a discharge ten times higher than normally. Data were collected under high QA criteria. Microplastics down to 20 µm were analysed using FTIR microscopy and a subsequent automated analysis of generated data. Here, microplastic numbers, shapes and masses of identified microplastics are presented.

Keywords: microplastic, riverine distribution, WWTP effluents

\*Speaker

# Laboratory and numerical testing of transport mechanisms of microplastics in rivers

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The anthropogenic pollution of streams and oceans with plastic is rising steadily. More than 335 million t of plastic are produced annually, of which approximately ten percent end up in the aquatic environment, where the plastic accumulates due to its high persistence. Especially microplastics pose a health threat to the aquatic fauna, because harmful substances from the surrounding water adhere to the small particles. Organisms tend to ingest those particles with their food and thus enable the pollutants to bioaccumulate in their fat tissue and to move up the food chain. Therefore, further input of plastics into our environment should be minimized and microplastics that are already in the aquatic system should be removed. A basic requirement for the latter is a detailed knowledge of the physical transport mechanisms of microplastics in rivers and oceans. Although microplastics accumulate predominantly in the oceans, streams act as major transport pathways. As particle properties of microplastics vary significantly depending on the polymer's type and origin, their transport processes within the river's flow regime may vary as well.

Therefore, the effect of microplastics' varying particle properties (density, diameter, shape and concentration) on their behavior in the water will be examined by subdividing the transport mechanisms into sedimentation, erosion, transport and infiltration. A combination of laboratory and numerical testing will provide a better understanding of the transport processes as well as it will give new insights regarding the fate of microplastics in rivers. As the research started in January 2018, solely the study design and some preliminary results on the sedimentation behavior of microplastics can be presented yet.

Keywords: transport mechanisms, particle properties, freshwater

\*Speaker

# Numerical modelling of microplastic transport in Weser estuary and Wadden Sea

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Microplastic (MP) is referred to disposed plastic particles, which are in the size of smaller than 5 mm. The main sources of microplastic contamination are the transported microbeads through the outflow of wastewater treatment plants into rivers and coastal areas. In contrast to the extensive researches about the environmental effects of MP on marine organisms, the quantitative studies on MP-transport mechanisms along rivers and estuaries are rare. To fill this knowledge gap and understand the MP-distribution, -transport and -accumulation mechanisms, a scientific research program was planned to study and develop the measuring approaches as well as numerical tools about MP contamination in Weser river and Wadden Sea of Germany, which is well-known as PLAWES. One of the main objectives of PLAWES is to provide a chain of systematic tools to manage and predict the MP fate from sources to receivers worldwide. To this end, the numerical studies of PLAWES for MP-contamination in river and estuary are performed through a coupled hydrodynamic model (using Delft3D-FLOW) with water quality and ecology module (using Delft3D-WAQ). The model results are calibrated and validated through performed measurements within the first successful measurement campaign in April 2018 by PLAWES-research group. More information about the numerical results and model approach will be presented in the conference.

Keywords: microplastic, numerical modelling, water quality

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# Giving value to plastic bottles: an effective solution for the top ocean polluter.

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*1 Retorna – Spain*

The European Union, in line with international organizations, has finally admitted the catastrophic effect of plastic on the environment and is in the process of enacting urgent legislation to curb this. Ten products have been identified as constituting the most recurrent in coastal and sea clean ups. The first item on the list is bottles, caps and lids, which will become part of the microplastics polluting the oceans<sup>1</sup>. A recent study reveals that 10 different types of plastic have been found in the intestine of every human analysed<sup>2</sup> and in Spain, 69% of marine food is infected by microplastics<sup>3</sup>. The Chinijo archipelago, in the Canary Islands, it is a perfect example of the harm that plastic can cause to both our environment and our health<sup>4</sup>. The proposed directive by the EU aims at a 90% collection rate and a 35% recycled content target, and identifies deposit return systems as the best way to achieve this. A growing list of countries is studying this measure, along with a series of bans and taxes, to curb the stream of waste flowing into the oceans. Bottle deposit laws are already in place across the world and have significantly reduced litter and increased the percentage of containers recycled. Other EU countries and Spanish regions are also on the way towards this solution, due to the increasing "on the go" consumption and littering of plastic bottles. The presentation analyses DRS from different perspectives; consumer, municipal, retailers, local and small fillers and from the labour perspective. The conclusion reached is that DRS is an effective, viable, consumer friendly solution for one of the top polluters.

Keywords: Deposit, refund system

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# Plastic input from the Seine River into the oceans: first results from the MacroPLAST project

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Most recent studies state between 0.1 and 4 Million t/yr of plastic enter the Ocean each year, most of it being transported by rivers. Those global estimations are associated with great uncertainties due to methodological difficulties to accurately quantify fluxes of plastic from continent into oceans. In addition, only few studies at the basin scale exist and no standard methods are applied to quantify those plastic fluxes. In this context, the Ministry of the Ecological and Solidarity Transition (France), in response to European directives, has initiated the MacroPLAST project to quantify macroplastic input from the Seine River into oceans. But, most importantly, the aim of this study is to develop a methodology replicable in other rivers. Different methods are investigated including modeling, field approaches, cameras, nets, etc. In this presentation, modeling and field methods using statistical data, floating booms or data from association collecting plastic litter are presented. The modeling method is based on Jambeck's approach applied to the Seine basin. Statistical and field approaches give similar order of magnitude, i.e. 1800-6300 t/yr entering the oceans from the Seine River (Figure 1). But, they are associated with large uncertainties and rough extrapolations. To avoid such extrapolations and statistical weaknesses, a new methodology has been developed and is actually in progress. It focuses on a probabilistic approach combining the tracking debris using GPS, tagged plastic litter and data from plastic litter collection along the riverbanks of the Seine estuary operated since 2008. First results at the mid of June 2018 lead to fluxes of 800 t/yr, an order of magnitude lower than the two other approaches. The results will be progressively improved as long as new data will be available and by investigating the potential hiding the plastic flux within the water column, below the water surface.

Keywords: Macroplastic, River, Flux. \*Speaker



## **November 20th – Panel 6.1: 10h30 - 12h30 (AGH), Panel chaired by Juliana-A. Ivar-do-Sul.**

### **Microplastic pollution of the Akyatan Beach one of the most important sea turtle nesting area at NE Mediterranean Coasts of Turkey**

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Plastic pollution is widely recognized as an important issue for marine environment. Currently, a large proportion of the plastics found in the marine environment are in the form of microplastics (< 5 mm). Microplastics are of most concern because they cannot be easily spotted, and are easily ingested by marine organisms and make their way through the food chain. Akyatan Beach is one of the most important green turtle nesting grounds in the Mediterranean with almost 50% of overall nesting occurring at this site. Loggerhead turtles also nest on this beach but in many fewer numbers. The beach is located on the eastern Mediterranean coast of Turkey and is almost 22 km in length. The aim of this study was to investigate, the abundance, composition and distribution of microplastics of Akyatan Beach. For this purpose, sediment samples were collected in May 2018 at nine sampling points using 1\*1 m quadrats. About 1000 g sediment with three replicates were taken and dried at 70°C. Microplastic particles were then selectively extracted from the sediment by floatation in a sodium polytungstate solution of 1.8 g/cm<sup>3</sup>. Microplastics were classified by color, shape, size and type. The results show that an average of 59.78±18.85 particles per kg (629.43±352.6 particles per m<sup>2</sup>) collected at all nine sampling points. The most abundant type of microplastics were fragments and the most abundant size were 1-5 mm size groups.

Keywords: Sandy Beaches, Sea Turtle, Microplastics, Mediterranean Sea

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## **Marine litter monitoring in the Neva Bay (Baltic Sea): first results**

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The problem of marine litter pollution has been investigated for the first time for the Neva estuary in the Russian part of the Gulf of Finland - an enclosed and highly populated water body at the easternmost tip of the Baltic Sea. Neva River is the largest river in the Baltic region with an annual discharge of 2 500 ub.m. River estuaries being unique aquatic ecosystems serve as an accumulation spot for all types of wastes, including plastics. The metropolitan area of St.Petersburg (largest city in the North-West of Russia) with the total population over 7 000 000 of permanent residents produces annually about 112 000 tons of plastic wastes; much of it finds its way to the adjacent waters. At the same time the water area of the Bay can serve itself as a source of beach pollution by plastic particles released from bottom sediments during repeated dredging works in the Neva Bay. Macro-, meso- and some micro-litter accumulation patterns were investigated during the monitoring campaigns of Russian State Hydrometeorological University in summer 2018 at 15 spots at sandy beaches of the Neva Bay and the Eastern Gulf of Finland, including urban and rural spots, using in parallel several European methods of beach litter monitoring. Results showed that there are differences in accumulation of marine litter in the northern and southern shores of the Neva Bay; however, some common patterns also were observed. Main types of litter were glass, fabrics, cigarette butts, and plastics. Analysis of local beach and coast types made it possible to discuss the most appropriate monitoring methods. At the same time some adaptations and modifications of existing European methods of beach litter monitoring are suggested considering regional specific meteorological (storm surges), hydrographical and geomorphological processes. Project was funded by Russian Foundation for Basic Research (18-55-76001 ).

Keywords: beach litter, Gulf of Finland, Baltic Sea, monitoring methods

\*Speaker

## Microplastics pollution on the beaches at the northern Black Sea coast during the monitoring of beaches sediments in 2016–2018

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During the monthly monitoring the study of qualitative and quantitative composition of microplastics on two urban beaches of Sevastopol (Omega and Uchkuevka) is conducted. Such studies were carried out on the northern Black Sea coasts for the first time.

Simultaneously with microplastic the granulometric composition of the beaches sediments was studied, to analyze the role of sediments size in spatio-temporal distribution of microplastic in underwater and above-water parts of beaches.

The samples were collected during 2016–2018 from the top 5 cm of the numerous square areas (1x1 m) placed at the four, 20 m-long transects located perpendicularly to the 100-meter-lines along the shore. Three types of stainless steel sieves were used: with the mesh sizes of 5 mm, 1 mm and 0.3 mm. In the laboratory, the collected sediments were put into a glass tank with a high concentration of sodium chloride (NaCl) 140 g·l<sup>-1</sup>. The floating plastic particles were recovered, sorted and categorized by type, usage and origin.

The samples for granulometric composition were collected on the water boundary, underwa- ter at depth 1, 2, and 3 m, and on the beach at distances 10, 20, and 30 m from water boundary. The samples were dried and sieved through 10 mesh form 0.1 mm to 10 mm. The relative abundance of every sediment fraction was calculated.

The highest microplastics parameters are registered on Omega beach in May 2017 ( $6.9 \pm 0.3$  items·m<sup>-2</sup>) and on Uchkuevka beach in August 2016 ( $3.5 \pm 0.1$  items·m<sup>-2</sup>). The minimum were on Omega beach in Januare 2017 ( $3,0 \pm 0,17$  items·m<sup>-2</sup>) and on Uchkuevka beach in May 2017 ( $1,5 \pm 0,054$  items·m<sup>-2</sup>).

The main sources of microplastic on the beaches are find out. Our data provide baseline knowledge for designing the monitoring and modeling strategies in the Black Sea.

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Keywords: Microplastics pollution, granulometric composition, beaches sediments, the Black Sea

## Accumulation of microplastics on sandy beaches of Mediterranean islands as an effect of tourism

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The BLUEISLANDS project brings together 14 partners from 8 countries in a systematic effort to properly identify, address and mitigate the effects of the seasonal variation of waste generation as an effect of tourism on mediterranean islands. One of the main activity of the project is to assess the accumulation of marine litter, in general, and of microplastics, in particu- lar, on sandy beaches of 8

Mediterranean islands (Mallorca, Sicily, Rab, Malta, Crete, Mykonos, Rhodes and Cyprus). For each island three different beaches were selected for comparison: one very popular and touristic beach, one popular beach but mainly used by locals and one remote beach. For each beach, 4 sampling campaigns were conducted in 2017: 2 during the low season and 2 during the high season. For each campaign, 5 samples of sand were collected (3 in the middle of the beach and 2 in the high water mark): the top 2-3 cm within a quadrat of 20x20cm were removed and stored into clean glass jars for further analysis. The samples are now in the processing phase (extraction and characterization of the microplastics). The first results show that, during the low season (February – April 2017) all the islands and beaches tested are affected by microplastics pollution. Their concentrations range from 10.3 particles/kg of dry sand on the remote beach of Cyprus to 809.6 particles/kg of dry sand on the touristic beach of Malta. The concentrations found on the touristic beaches are always higher than those found on the remote beaches. Once obtained, the final results for the 4 campaigns will be compared to the accumulation of marine litter on the same sites and the number of tourists in order to assess the effect of tourism on the generation and the accumulation of microplastics.

Keywords: Microplastics, tourism, beaches, BLUEISLANDS

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## Microplastic pollution on Gran Canaria island beaches

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In recent decades, plastic pollution in the ocean has increased exponentially. With an estimation of more 270 thousand tons of plastics floating in the marine environment where they tend to migrate to the oceanic margins, accumulating in convective zones. The Canary Islands, located on the eastern margin of the Atlantic Ocean, are an obstacle to the Canary Current. There, it is of great interest to study the amount and type of plastic that migrates around the archipelago, as well as the proportion of plastic that washes up on the coast. This study of microplastic pollution on Canary Island beaches is a starting point for seasonal monitoring of plastic waste and future research that will aim to explain the consequences that this marine litter can have on marine ecosystems. The majority of the items observed were fragments from bigger plastic objects, more of the 50% of the items sampled. Even, the transparent resin pellets or nurdles, the semispherical items used as raw material in the production of plastics, were found on most beaches and showing a 14% of the total microplastic pollution. The Canary Islands do not have a plastics industry, so the origin of this

marine debris is due to ocean circulation. On the other hand, microfibers were sampled too, showing a mean maximum concentration of 2000 items/ m<sup>2</sup>. The distribution of microfibers is totally different from that of larger microplastics and mesoplastics, suggesting a possible endogenous origin of the contamination, probably by wastewater discharges, ravines and beach users.

\*Speaker

## Quantity and type of plastics in the beaches of the region of Murcia (SE Spain): towards a monitoring of marine litter in the marine spatial planning

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During the last decades much attention has been paid to marine litter pollution, mainly due to plastics and microplastics. The Descriptor 10 of the Marine Strategy Framework Directive (2008/56/EC) is devoted to plastics in particular and marine litter in general. So there is a need to control the quantity and type of plastics present in the marine environments.

Among these environments, beaches are one of the most impacted areas due to plastic pollution but studies about content and type of plastics in Spanish beaches are scarce. There is a need to implement a monitoring programme both in beaches but also in coastal waters for these emerging contaminants.

In the present study, the amount and type of plastics present in several littoral sandy areas from the Region of Murcia (SE Spain) are presented. In addition, the amount of a regulated contaminant such as Hg in the plastics is also shown. The problem of these plastics is not only physical, but also associated to the pollutants contained in this material during its production or adsorbed once the plastics are released to the marine environment. Recent studies have demonstrated that once in the environment, plastics are able to adsorb metals and concentrate them (Ashton et al., 2010; Holmes et al., 2012). This fact can have several environmental and ecotoxicological implications as the adsorption-desorption kinetics of metals and plastics under different environmental conditions (i.e. pH, salinity or temperature) will release these metals from plastics to the water column or, in contrast, will act as metal carriers (Brennecke et al., 2016) to the trophic chain, entering through the filter-feeder animals for example.

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Keywords: Plastic, metals, pollution, marine environment, spatial, planning.

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## The french approach for msfd beach microplastic monitoring : presentation and first results

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The descriptor 10 of the Marine Strategy Framework Directive (MSFD) requires that the “properties and quantities of marine litter do not cause harm to the coastal and marine environment.” To achieve or maintain Good Environmental Status (GES) in European Seas, European Member States (MS) have to develop strategies that should lead to programmes of measures. This involves the establishment of monitoring programmes, enabling the assessment of the status of marine waters on a regular basis. As French national MSFD scientific pilot for the indicator D10C2 beach microplastic, the Centre of Documentation, Research and Experimentation on Accidental Water Pollution (Cedre) is in charge of the French beach microplastic monitoring program. Considering that microplastics could be either deposited on or buried in the sand, two protocols have been developed. The first, based on recommendations of the MSFD Technical Subgroup on Marine Litter (Galgani et al., 2013), targets buried microplastics (0.1 – 5 mm) and involves sand sampling and subsequent microplastic extraction and analyses in lab. The second one is optional as only applied in presence of stranded patches or “slicks” of microplastics (1 – 5 mm); based on a protocol commonly used for oiled shoreline survey during an oil spill, it consists in calculating the volume of microplastics by visual assessment. In this communication, the French approach for MSFD beach microplastic monitoring will be presented as well as first results obtained for some French beaches.

Keywords: Microplastic, beach, MSFD, monitoring

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## **November 20th – Panel 6.2: 10h30 - 12h30 (EC), Panel chaired by Martin Wagner.**

### **Effects of microplastics presence on different developmental stages in invertebrate species**

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When microplastics (MPs) reach the marine environment, they can interact with a wide range of organisms. The main physical threats for marine species that ingest MPs are damaging and blocking of the feeding appendages and digestive system and limiting of the food intake. Thus, we investigated the effects induced by the presence of spherical polystyrene MPs particles on different developmental stages in the invertebrate species *Ciona robusta*, *Clavelina lepadiformis* and *Paracentrotus lividus*. The chosen species offered the unique opportunity to evaluate and compare the impact of MPs on the development of animals with different feeding strategies: the pelagic suspension feeding plutei and the sessile filter-feeding ascidian juveniles and adults. We tested four different MPs concentrations based on available environmental data. Both ascidian juveniles and plutei were able to ingest MPs beads with different feeding efficiency at all the concentrations tested. In ascidians, survival rate of larvae and juvenile was not influenced while metamorphosis was the process most affected by microplastics. The percentage of individuals that completed metamorphosis was statistically lower in all the exposed groups, compared to control. In sea urchin plutei, the body shape was altered after ingestion of MPs. Adults of *C. lepadiformis* filtered a huge amount of water and quickly ingested MPs also at the lowest concentration tested. Our results revealed that developmental stages of different species are highly sensitive to MPs presence, prompting the necessity to monitor coastal invertebrate populations since MPs can alter generation recruitment.

Keywords: polystyrene, sea urchin, ascidian

\*Speaker

# The impact of microplastic ingestion on the behaviour of the Common Periwinkle *Littorina littorea*

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The common periwinkle (*Littorina littorea*) is an intertidal gastropod of commercial importance. Consumed domestically and exported internationally, it is a species of great value to the Irish seafood industry. Ecologically, the species is regarded as a keystone grazer on rocky shores, where it reduces algal cover and allows space for other organisms to colonise. However, through this grazing activity, the common periwinkle has been shown to ingest microplastic fibres and beads from the surface of algae. Despite evidence of this phenomenon, no study has yet explored the behavioural effects of microplastic consumption in the species. The goal of this study is to collect baseline data on microplastic levels, and to explore possible behavioural implications of microplastic ingestion in the common periwinkle. Preliminary results show that 99% of microplastics consumed by Irish periwinkles are in the form of fibres and that microplastic levels are similar between clean and contaminated sites (1.92 & 2.02 fibres/individual respectively). Future research will assess a number of behavioural responses to microplastic ingestion, including feeding, movement rate, and predator avoidance. This work has important implications both ecologically and commercially. As the common periwinkle is consumed by humans, it is vital that microplastic levels in harvested populations be quantified and monitored long term. Ecologically, microplastic ingestion by periwinkles could have a number of negative implications, both at the species and community level. Potential reductions in movement speed, grazing capacity, or ability to evade predators could all affect the ability of periwinkles to regulate algal cover, and thus maintain succession. In turn, this could have negative consequences for the equilibrium of rocky shore communities. This study provides the first data on microplastic levels in Irish periwinkles and will provide the first study into the behavioural effects of microplastic ingestion in the species.

Keywords: Microplastic, Gastropod, Behaviour

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# Food quality and microplastics alter feeding behavior and digestive enzyme activities in marine isopods

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Ingestion of microplastics can impair nutrition of marine invertebrates. In a laboratory study, we tested whether microplastics affect ingestion rates and gastrointestinal enzyme activities in the marine isopod *Idotea emarginata*. Isopods were exposed for eight days to one out of four different food treatments: natural food (the brown alga *Fucus vesiculosus*) or synthetic food consisting of freeze-dried algal powder embedded in agarose, both, with or without microplastic particles at concentrations of 40 items per mg of food. The isopods accepted both types of food but consumed significantly more (average 3.7-fold) of the agar based synthetic food. *I. emarginata* responded to the reduced content of digestible organic matter in the synthetic food by a compensatory adjustment of the ingestion rates. Addition of microplastics had no effect on feeding rates. Apparently, the influence of microplastics on the food quality is negligible compared to effects of nutrient deprivation. Digestive enzyme activities were only marginally affected in the approach with natural food. In the approaches with synthetic food, however, activities of unspecific esterase increased in an organ-specific manner. Animals which were fed with synthetic food alone, showed elevated esterase activities in the guts while animals, which received synthetic food blended with microplastic showed elevated esterase activities in the midgut glands. The results suggest that microplastics do not significantly affect digestive processes as long as sufficient nutrients are available. Under nutrient limiting conditions, however, microplastics seem to generate combined effects with so far unknown consequences.

Keywords: Marine invertebrates, isopods, feeding, food quality, enzymes, combined effects

\*Speaker

# Do synthetic and natural microparticles cause similar cellular effects in marine invertebrates?

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In coastal areas, and especially in estuaries, organisms are constantly exposed to a variety of

suspended particles. The particles are mostly of natural origin, i.e. fine sand grains, diatoms shells, cellulose fibres or chitinous fragments. Since several decades, however, man-made synthetic plastic particles are accumulating in the environment. The later are of great interest in environmental and toxicological sciences as they are suspected to induce cellular modifications and stress in organisms upon ingestion. This situation raises a number of questions whether natural and synthetic microparticles induce similar effects and damage in cells and organisms. In order to investigate this issue, marine organisms with different feeding modes will be subjected to natural and synthetic particles. Blue mussels (*Mytilus edulis*) and brown shrimps (*Crangon crangon*) will be collected from the field, acclimatized to laboratory conditions, and exposed to natural and synthetic particles for 6, 24, and 48 hours. Microscopic analysis will be done to localize the particles in digestive organs. Histochemical and biochemical analyses will be used to detect stress markers in cells and tissues. The goal of this doctoral project is to understand the hazardous effects of microparticles and to differentiate between anthropogenic and natural items. The findings will be beneficial in estimating the actual hazard potential of microplastics and in defining actual threat boundaries for marine invertebrates.

Keywords: Crustacea, Bivalvia, Oxidative Stress, Histochemical and Biochemical Analyses

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## Zebrafish exposure to high-density polyethylene and polystyrene microplastics: effects on liver transcriptome and gastrointestinal histology

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Due to the constant increase of plastic use and production, microplastics (MPs) have become a contaminant of serious concern for the aquatic environment. However, the biological pathways affected by the exposure to different MP polymers still needs to be elucidated, in particular at the "omic" level. The present study focuses on the identification of the molecular pathways affected by the exposure of zebrafish (*Danio rerio*) to different concentrations of a mixture of two environmentally relevant MPs. Adult zebrafish were fed daily with dry fish food (control group, N=12) and food supplemented with a mix of pristine high-density polyethylene and polystyrene microplastics (two experimental groups: 0.1 and 1 mg/L, N=12 each) for 20 days. The MPs

dimension ranged from 25  $\mu\text{m}$  to 90  $\mu\text{m}$  for both polymers.

At the end of the exposure period, the fish liver was dissected, and its whole transcriptome was analyzed by next-generation RNA-sequencing technologies on an Illumina platform. In addition, the gastrointestinal tract and the gills were dissected and fixed for histology and immunohistochemistry. The exposure to polyethylene and polystyrene microplastics affected the liver transcriptome in a dose-dependent way, inducing the differential regulation of specific suites of genes compared to the control. Histological analyses evidenced changes in the inflammatory response occurring at the two mucosal tissues selected for observation in the MPs treated fish. The correlation of histological alterations with differential gene expression could represent a useful approach to decipher early warning for effects at higher biological organization. This study provides a comprehensive transcriptomic dataset, useful to understand a wide range of biological pathways affected by MPs exposure as a model for studies on fish also in the field.

Keywords: microplastics, polyethylene, polystyrene, zebrafish, liver transcriptome, histology

\*Speaker

## Bioaccumulation and gut microbiome response in the great pond snail *Lymnaea stagnalis* exposed to PBDEs in the presence and absence of nylon microplastics

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It is recognised that microplastics will associate with persistent organic chemicals within the environment, potentially changing their bioavailability and the interaction of organisms with these chemicals. The aims of this study were to investigate how the presence of microplastics affect PBDE bioaccumulation and the gut microbiome in the great pond snail *Lymnaea stagnalis*. Snails were exposed to microplastics and PBDEs independently and in combination for 96 hours. Microplastic particles (13-15  $\mu\text{m}$  nylon powder) were mixed with quartz sand sediment at 1% w/w. A PBDE mix (containing BDE-47, 99, 100, 153 and PBB-153) was added to the sediment-microplastic mix in glass vessels at six environmentally relevant concentrations (94, 188, 375, 750, 1500, 3000 ng g<sup>-1</sup> each PBDE), with six replicates consisting of one snail per treatment. There was no significant

mortality observed in any of the treatments after 96 hours exposure. Of the six snails per treatment, three were analysed for PBDE tissue concentration and three were analysed for gut microbiome composition. The presence of microplastics did not significantly change PBDE bioaccumulation. However, our data indicate that the presence of microplastics did influence gut microbial community diversity. In snails exposed to microplastics only (without PBDEs), microbial diversity was lower compared to those not exposed to microplastics (Fig. 1). In the presence of microplastics, with increasing PBDE concentration, gut microbial diversity increased. In comparison, when microplastics were absent microbial communities were less diverse at the higher concentrations (Fig. 1). A similar trend is reflected in the community composition data. These data imply that microplastics alone can influence the gut microbial community and they can also alter the effects of PBDEs on the gut microbiome. Given the importance of the gut microbiome for nutrition, metabolic function and immunity, such perturbation to the microbial community may have implications for organism health and fitness.

Keywords: Great pond snail, freshwater, ecotoxicology, PBDEs, organic chemicals, POPs

\*Speaker

## TROPHIC TRANSFER OF MICROPLASTICS FROM COPEPODS TO PLANKTIVOROUS AND THEIR IMPACTS

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Microscopic plastic (MPs) debris, termed "microplastics", are of increasing environmental concern. Recent studies have demonstrated that a range of zooplankton, including copepods, can ingest microplastics. Copepods are a globally abundant class of zooplankton that form a key trophic link between primary producers and higher trophic marine organisms. The increased widespread occurrence of MPs corresponds to growth in the manufacture of plastic materials and include sources such as cosmetic exfoliates, polyester fibres from fabrics, polyethylene fragments from plastic bags, and other larger plastic items. Since microplastics occupy the same size range as many planktonic organisms they can easily be mistaken for food and thus may affect a wide range of

marine organisms, including copepods. Especially the planktivorous fish may erroneously capture microplastics that resemble their evasive copepod prey. The consumption of microplastic debris can result in gut blockages, growth, heightened immune response, loss of lipid reserves and significantly reduced algal feeding in the copepod. The potential risk to food security and thereby human health has led regulators to call for better understanding of the fate and effects of microplastic debris on marine life. These studies provide fundamental information on the ingestion and biological effects of microplastic debris upon copepods, knowledge of which is important given the key role that copepod play in the transfer of microplastics to higher trophic levels and, thus, ecosystem function. These findings provide pathways for further research and highlight the influence that feeding strategy and prey selectivity may have in determining the negative effects associated with microplastic uptake.

\*Speaker

Keywords: Marine copepods, Microplastic, Feeding, Ecotoxicology, Food web.

## **November 20th – Panel 7.1: 14h - 15h30 (AGH), Panel chaired by Tania Montoto.**

# PHOTOCHEMICAL FRAGMENTATION OF FRESHWATER (MICRO)PLASTICS UNDER UV IRRADIATIONS

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We begin to understand and describe more and more the fate of a plastic waste arriving (and remaining) in the aquatic environment. Nevertheless, we still do not know many things, for example, the time scaling of the process from the abandonment of a waste, its arrival, and its persistence in the aquatic environment. During this period, the material will be exposed to various environmental aggressions that will initiate and spread the photo aging of the material. This scenario is accompanied by a physical fragmentation into particles of increasingly smaller sizes, and by a chemical functionalization due to the photo-oxidation of the macromolecular chains. Finally, the increase in both the specific surface area and the chemical functionality may influence strongly the interaction parameters with persistent organic pollutants.

We have studied, in simulated laboratory conditions, the fate of various plastics fragments (Polystyrene, Polypropylene and Polylactic Acid) immersed in fresh water and UV irradiated. We worked either with real wastes (from post-consumer sector) or with model polymers totally free of additives. The polymers were chosen for their different physical properties. Polystyrene behaves like a glass ( $T_g = 104 \text{ }^\circ\text{C}$ ) at the temperatures of use, which is not the case of the PP ( $T_g = 0 \text{ }^\circ\text{C}$ ). Finally, PLA can start hydrolysis reactions.

During the exposure time, solids and liquids (a small volume of water) are taken for analysis (melt rheology, ion and liquid chromatography). The main result is that in all cases there is a formation and leaching of short chain (1C, 2C, 3C) carboxylic acids (acetic and formic acids, lactic and glycolic in the case of PLA) all known to be markers of polymer degradation. In the absence of light no transfer of any acid in the liquid is recorded.

Keywords: microplastics, Oxidation, ageing, chemicals

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# Influence of environment and polymer morphology on fragmentation and production of micro/nanoplastics

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Weathering of non biodegradable polymers in the environment is a sum of complex phenomena such as oxidation, hydrolysis and degradation governed by photo/thermal processes [1]. These phenomena strongly affect all physico-chemical properties of the polymer : chemical composition [2], crystallinity [1], wettability [3] leading to their fragmentation in microplastics. To provide a better understanding of these processes and to predict the evolution of microplastics environmental concentrations, laboratories experimentations are needed. "Additive free" polyethylene (PE) and polypropylene (PP) films were blown extruded in order to control their morphologies and thicknesses and were placed during several months in an accelerated weathering chamber. During the weathering process the physico-chemical properties of the films were regularly analyzed through spectroscopy and water contact angle measurements.

Despite a high oxidative degree, no fragmentation was observed for polymer films in air. In contrast, the films in the still Milli-Q water fragmented following distinct patterns and leading to different fragments size distribution. (cf Figure 1).

By combining Atomic Force Microscopy (AFM) and optical microscopy imaging, we were able to show that the fragmentation process strongly depends on the morphological structure of the film and on the water presence. Finally, although, nanofragments ( $< 1\mu\text{m}$ ) were not in sufficient quantity to be detected by light scattering measurements (DLS), surface erosion of the films at the nanoscale was confirmed for PP.

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Keywords: Fragmentation, Polymer Weathering, Microplastics, Nanoplastics

\*Speaker

# Characterization of nanoplastic aggregation as a function of temperature and ionic strength

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The wide range of applications, ubiquitous distribution and persistent nature of plastic has become a global concern. Large plastic debris gets disintegrate into micro or/and in nano- size range by a number of physical, chemical and biological factors in the environment. Its complexity due to the presence of additives, tendency to absorb other contaminants or propensity to act as a vector to cotransport pollutants with limited detection techniques has increased the concern in the scientific community. Although there are extensive pieces of literature on the occurrence of microplastic/nanoplastic debris in the environment, the aggregation behavior of nanoplastics that determines the fate and transport of these particles in natural aquatic condition has received very limited attention. A part of the study was to investigate the effect of varying temperature (15 0C, 25 0C, and 35 0C) on the rate of aggregation of polystyrene nanoplastics over a range of monovalent (NaCl) and divalent (CaCl<sub>2</sub>) salt concentration using time-resolved dynamic light scattering. The stability ratio and critical coagulation concentration (CCC) was calculated for both salts and the temperature ranges. The aggregation kinetics exhibit reaction- limited (slow) and diffusion-limited (fast) regimes for both NaCl and CaCl<sub>2</sub>. Moreover, high aggregation due to charge neutralization was observed in presences of low concentration of CaCl<sub>2</sub> followed by NaCl. However, at higher concentration of CaCl<sub>2</sub> charge reversal is observed that stabilizes the nanoplastic. In addition, with a decrease in temperature, a rise in stability ratio for the polystyrene nanoplastic was observed. Preliminary results indicate that the stability of nanoplastics is highly influenced by the presence of electrolytes and the temperature of the aquatic body.

Keywords: Nanoplastic, polystyrene, aggregation, stability ratio

\*Speaker



# Photodegradation of PE small microplastics : erosion vs fragmentation

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# Woven metal filter media for microplastics sampling from water

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Woven wire meshes represent an important category of filter media and are widely used in various filtration tasks. The fact that they are a pure metallic filter media makes them a prior choice for microplastic sampling from water as the filter material doesn't contaminate the specimen. Another advantage of a woven media is their geometrically determined pore shape and size, delivering a very sharp cut point when used in filtration. Filter media are commonly characterized by their opening. Unfortunately, this characterization by opening is not specific without knowing the conditions under which this value was actually determined. As most manufacturers test the opening of a woven media in different ways, the end user is subjected to very inhomogeneous and unstandardized characterization methods. This ultimately leads to very subjective interpretation of particle measurements conducted with woven wire meshes as filtration medium and results which are not comparable. Table 1 gives a short overview of pore sizes for a 200 µm mesh determined with different testing methods. This paper intends to show how this problem can be avoided by characterizing woven media by a standardized pore opening which is given by major suppliers of woven wire mesh. Its goal is giving an overview of existing media and helping the end user to select an appropriate mesh for microplastic sampling which then can be compared to other results which have been achieved with the same method.

*Keywords: Sampling, Filtration, Standardization*

*\*Speaker*

## An unexplored type of microplastics revealing a neglected way of plastic fragmentation found in the beaches of Hong Kong

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Microplastics in different environments vary in composition due to the variation of the sources and factors that distribute them. In order to characterise the types of microplastics in the beaches of Hong Kong, microplastics (size of 1 – 5 mm) were collected from 10 beaches. Out of 5000 pieces of

microplastics analyzed, over 50% (by pieces) were plastic fragments with a predominance of white or transparent (41%) and blue (28%) in colour. More importantly, a special type of fragment which has not been reported before is revealed. These special fragments, composing about 13% of the fragments collected, are interestingly triangular in shape with at least two of the three sides being characteristically straight and re-sembling a cut made by compression, often coupled with signs of tearing. Objective observations from some special pieces have made apparent differences between these "trimmed fragments" and those triangular fragments that were fractured randomly as the plastic degraded. In addition, species of fish were examined to investigate their ability to make these fragments by biting. Preliminary findings showed that some species of the Tetraodontidae are likely to be the causes. A brief review of photographs of microplastics from other parts of the world supported that the "trimmed fragments" discovered in this study could be widely distributed. If this type of "trimmed fragment" is confirmed to be formed by biological causes in macro-scale instead of natural weathering, the finding here would have wide implications on current ecotoxicological and modeling studies for microplastics since the active biting of large plastic debris has generally not been considered as a factor.

Keywords: Microplastic ingestion, source, fragments

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## **November 20th – Panel 7.2: 14h - 15h30 (EC), Panel chaired by Bethany Jorgensen.**

### **Freshwater plastic pollution: recognizing research biases and identifying knowledge gaps**

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The overwhelming majority of research conducted to date on plastic pollution (all size fractions) has focused on marine ecosystems. In comparison, only a few studies provide evidence for the presence of plastic debris in freshwater environments. However, owing to the numerous differences between freshwater studies (including studied species and habitats, geographical locations, social and economic contexts, the type of data obtained and also the broad range of purposes), they show only fragments of the overall picture of freshwater plastic pollution. This highlights the lack of a holistic vision and evidences several knowledge gaps and data biases. Through a bibliometric analysis we identified such knowledge gaps, inconsistencies and survey trends of plastic pollution research within freshwater ecosystems. We conclude that there is a continued need to increase the field-data bases about plastics (all size fractions) in freshwater environments. This is particularly important to estimate river plastic emissions to the world's oceans. Accordingly, data about macroplastics from most polluted and larger rivers are very scarce, although macroplastics represent a huge input in terms of plastics weight. In addition, submerged macroplastics may play an important role in transporting mismanaged plastic waste, however almost no studies exist. Although many of the most plastic polluted rivers are in Asia, only 14% of the reviewed studies were carried out in this continent (even though the major inland fisheries of the world are located in Asia's rivers). The potential damage caused by macroplastics on a wide range of freshwater fauna is as yet undetermined, even though negative impacts have been well documented in similar marine species. We also noted a clear supremacy of microplastic studies over macroplastic ones, even though there is no reason to assume that freshwater ecosystems remain unaffected by macro-debris.

Keywords: plastic pollution, freshwater environment, macroplastic, developing countries, endangered fauna.

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# Sampling microplastics in turbulent rivers using a stationary net from a bridge: what should be the sampling duration? How many samples are needed to reduce bias?

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Rivers are recognized as a major pathway for plastic entering the ocean; however, few studies have investigated microplastic inputs from rivers to the ocean. Moreover, there is currently no harmonized protocol or guidelines dedicated to microplastic sampling in rivers. This presentation aims to improve microplastic sampling in rivers and to bring answers to two important questions: what should be the sampling duration? How many samples are necessary to reduce bias?

Sixteen samplings were carried out April 6th, 2018. A 300 m stationary net was set up from a bridge downstream of Pau city, southwest of France, on the Gave de Pau river. Ten samples were collected with a sampling duration of 5 minutes, three with a sampling duration of 3 minutes and three with a sampling duration of 7 minutes. The macro fraction was removed with a 5.6 mm mesh sieve. The thinner fraction was then digested using a hydrogen peroxide oxidation. Microplastics were then counted under a binocular microscope. A subsample of the identified microplastics were tested through spectrometry FT-IR. Microfibers were excluded from the analysis.

Our sixteen samples show a mean density of  $3.34 \pm 0.20$  microplastics per m<sup>3</sup> with 61% being made of polyethylene. Statistical analysis didn't show any differences between mean microplastic densities for our different sampling durations. All three durations seem therefore relevant for a water speed surrounding 1.4 m/s. Our calculation shows that the collection of 11 samples is needed to divide by two the standard error. Considering that microplastic distribution is homogenous in the water column of turbulent rivers, the annual microplastic load of the Gave de Pau river is estimated at  $5.46E11 \pm 3.33E10$  fragments. Overall, this study contributes to the development of a methodology to sample microplastic in rivers. Such method is expected to facilitate data collection, comparison and model calibration.

\*Speaker

Keywords: microplastics, sampling, rivers, standard error

# Evaluating citizen science as a technique for spatial analysis in freshwater ecosystems: A case study in the Ottawa River watershed, Canada.

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A collaboration between Carleton university researchers and volunteers from the Ottawa Riverkeeper network provided a citizen science partnership to examine the spatial distribution of microplastics in the Ottawa River, Canada. Citizen scientists sampled 100 litres of river water along the Ottawa River and submitted samples to the Carleton University research team for analysis. 43 samples were received representing 23 sample locations across an approximate 600-kilometre length of the Ottawa River. The advantages of using citizen science collaboration for freshwater microplastic research include engaging the citizen scientists in the issue of microplastic pollution. The expansive network of volunteers through the Ottawa River valley enabled a relatively inexpensive method for data collection in comparison to a research team conducting the research. However, there are draw-backs to this method. For example, not all citizen scientists followed the specific directions, in addition some citizen scientists failing to conduct control samples. It is recommended future citizen science projects should consider an established network of volunteers. The current research had the advantage of utilising the Ottawa Riverkeeper and its volunteer base. Furthermore, it is recommended to continually convey the importance of correctly filling out standardised forms and conducting control samples to the citizen scientists. Lastly, developing a method of enabling the citizen scientists to sample more than 100 litres of water at their desired locations would be advantageous. This would help reduce the microfiber contamination influence on samples. However, it must be noted, if you ask citizen scientists to sample for an unreasonable amount of time, one could potentially decrease volunteer interest, as is important to keep the scientists actively interested and engaged in the research process for an effective and successful collaboration.

Keywords: citizen science, microplastics, Ottawa River, microfibres

\*Speaker

# Microplastic pollution in the River Trent UK, and the use of EASYLIFT to optimise microplastic recovery.

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Freshwater environments have not been extensively researched to determine the level of microplastic pollution. As freshwater systems will contribute to marine plastic pollution more research into freshwater microplastic research is needed. The River Trent is the third largest river in the UK, it passes through several major cities, it also runs through many nature reserves so the effect of microplastic pollution in the Trent could be far-reaching. This project aims to quantify the level of microplastic pollution along the duration of the river, by taking both 1-litre water and 132cm<sup>3</sup> sediment samples from twenty-five locations along the river. Sediment samples and water samples were obtained using the ‘grab method’ outlined in Barrows et al, (2017). The River Trent could then be characterised to determine how the concentration of microplastics will change as the river progresses. The microplastics recovered were characterised by factors including the type of microplastic, colour, length, width, cross-sectional shape and birefringence to determine the polymer type. The use of EASYLIFT as a method for optimising microplastic recovery was compared to previously used methods of manually searching for and retrieving fibres. Approximately 1333 microplastics were found over 28 samples and seven locations, an average of 6.2 microplastics/l-1 were found in the water samples and for the sediment samples, there was an average of 3.2 microplastics per 132cm<sup>3</sup> of sediment. Using EASYLIFT to recover the microplastics after they had been filtered recovered more microplastics than manually recovering them. In all instances when manual methods of recovery were used first EASYLIFT recovered at least one additional microplastic. Whereas when EASYLIFT is used first in most instances it recovered all the microplastics present, this means that using EASYLIFT to recover extracted microplastics could provide a more accurate indication of the level of microplastic pollution.

Keywords: microplastics, rivers, EASYLIFT, quantification, Trent, UK, pollution, freshwater, sediment, collection method, recovery

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# Effectiveness of distinct methods for isolation of microplastics in freshwater systems

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Plastics, one of the most demand material worldwide, are considered an emerging aquatic pollutant due to their ubiquity, high persistence and insufficient management. Especially, microplastics (< 5 mm) are of scientific and social apprehension as they can reach high densities, derive from a variety of sources and can interact with biotic and abiotic environment. Currently, the concerning about microplastics (MPs) in freshwater systems has been increasing, notwithstanding there is no unified method for MPs isolation in these systems. This result in inaccurate data that differs in quality and resolution, not allowing data comparison between different studies. Hence, this work aimed to assess the effectiveness of distinct separation methods as an attempt to identify and establish the most cost-effective method. For that, artificial samples containing eleven plastics belonging to the most common types of polymers (e.g. low/high-density polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate) were prepared and subjected to distinct methods. These methods included the single use of distinct density separation methods (using sugar, olive oil and zinc chloride), as well as organic matter degradation methods using hydrogen peroxide (wet peroxide oxidation) and multienzymatic detergent (enzymatic digestion), both combined with separation methods. The samples were then undergoing the detection, quantification and identification of polymers using a dissection microscope and Fourier transform infrared spectroscopy (FTIR). Several criteria were considered in order to achieve the aims of this work: effectiveness of density separation and organic matter degradation, the total mass of polymers recovered, cost of each procedure, the time spent, the simplicity and the quality of recovered polymers. Based on this multi-criteria approach, this study concludes that the wet peroxide oxidation with addition of zinc chloride was the most cost-effective method, and therefore their use is recommended in MPs monitorization studies in aquatic systems.

Keywords: Microplastics, freshwater systems, isolation methods

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# Toward automated plastic detection in rivers

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The majority of marine macro plastic is transported from rivers. Current estimations are based on modelling approaches, because of the lack of measurements. On-site river observations are necessary to improve our understanding of macro plastic behavior. Most of the current measurement methods rely on labor and equipment intensive methods. To quickly expand the on-site measurement dataset, The Ocean Cleanup is developing an automated monitoring tool based on camera footage from bridges. From these images we can derive plastic particle counts, organic particles and flow velocities. This system was currently tested under varying conditions, on several locations. Application of this monitoring method is currently used as an expansion to current applied measurement methods. It is most suited to better understand long-term variability and time dependent variables. Now, expansion of the amount of camera monitoring locations could greatly improve the understanding of plastic outflux estimations. The video dataset allows for several post processing possibilities. In the future, the applications of camera imagery can be refined with more optimized processing algorithms and applied to the currently created dataset.

Keywords: riverine, plastic, macro, automated, detection, cameras, photo, video, footage, machine vision, monitoring, processing algorithms

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## **November 20th – Panel 8.1: 15h45 - 17h30 (AGH), Panel chaired by Matthew Cole.**

### **Impacts and implications of plastic adhesion to corals surface**

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Despite thousands of tons of plastic dumped yearly in the Red Sea, its surface waters support one of the lowest concentrations of litter estimated worldwide. Given the extension of coral reefs in the basin, we hypothesize a major role of reef organisms as corals in retaining smaller plastic particles (<0.5 mm) missing in surface waters. While ingestion of plastic in corals has been demonstrated, it is not clear whether environmental conditions as presence of the natural prey affects or enhances plastic feeding rates. To this end, we conducted experiments with 3 coral species common in the Red Sea (*Pocillopora verrucosa*, *Acropora hemprichii* and *Goniastrea retiformis*), exposed to either plastic beads (sizes 53 to 500 µm), *Artemia* shrimps or a mix of the two. During the experiment, we observed a significant logarithmic decrease of plastic in the water due to adhesion of beads to the surface rather than ingestion, showing that the three-dimensional structure of the coral is a sink for microplastics. Plastic ingestion was recorded in all specimens and correlates with amount of plastic adhering to the animal surface, suggesting adhesion as a key mechanism to deliver plastic to the polyp. This experiment was not able to show an influence of the natural prey on the plastic ingestion, result that was masked by the more relevant retention of microplastics by the coral surface. We indeed estimated that studied corals can sink up to 50% of the plastic present in the water column, in a species-specific extent dependent on the complexity of their skeleton structure.

Keywords: Microplastic, Ingestion, Corals, Sinks, Red Sea

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## **Bioavailability and ingestion of microplastic by zooplankton in the natural environment**

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Microplastics have been documented in marine environments worldwide where they pose a potential risk to a range of biota. Of particular susceptibility are zooplankton, small ubiquitous marine animals that provide an essential link between primary producers and higher trophic levels (e.g. commercial fish species). Laboratory studies have established that zooplankton, such as copepods, readily ingest microplastics, and that such ingestion decreases their energy budget and negatively impacts reproduction, health and survival. However, sampling, classifying and enumerating the size range of microplastics readily consumed by zooplankton, and understanding the extent to which zooplankton encounter and ingest plastic particles and fibres within their natural environment is imperative, not least as there is potential to disrupt the link between primary producers and higher trophic levels, and for trophic transfer and bioaccumulation of microplastics within the marine food web. In this study, we compared the amounts of microscopic anthropogenic debris collected using 100, 335 and 500  $\mu\text{m}$  aperture nets, across 14 sites in the western English Channel, to determine if sampling with traditional 335  $\mu\text{m}$  aperture nets underestimates microplastic. Our findings show that as mesh size decreases, the number of anthropogenic microdebris items collected increases. Furthermore, the mean size of items collected was smaller in nets with a smaller mesh size.

We also sampled six sites in the western English Channel over the course of one year to determine the types and amount of anthropogenic debris ingested by zooplankton in the natural environment. Our results demonstrate that zooplankton routinely encounter and ingest microplastics under natural conditions. Encounter rates resulting from ingestion ranged from 1 particle/every 6-125 zooplankton. The incidence of ingestion of different zooplankton at different temporal and spatial locations and potential impact on the health of the population and higher trophic levels will be discussed.

Keywords: microplastic, zooplankton, mesh size, English Channel

\*Speaker

## Mechanisms and effects of microplastic ingestion by planktivorous fish

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Millimeter-sized plastic particles (microplastics) have been documented in many fish species from most marine environments, but the factors influencing the ingestion still warrant to be tested to better understanding microplastic pathways within trophic webs. The comparison of microplastics and planktonic organisms in surface waters and in the digestive tract of juvenile planktivorous fish captured along the coast of Easter Island revealed that fish ingested preferentially blue microplastics similar to their common copepod prey. Experiments in the laboratory confirmed that visually-oriented planktivorous fish capture mostly microplastics resembling their food. Moreover, microplastics were only swallowed when co-captured with food, and were otherwise spit out, suggesting that food produces a 'gustatory trap' that impedes the fish to discriminate and reject inedible particles. Fish might thus ingest accidentally marine-weathered microplastics because they are overgrown by a biofilm that alters the gustatory response of the fish. Experiments in the laboratory will test whether juvenile fish ingest preferentially marine-weathered over unweathered microplastics, and also examine potential impacts of microplastic ingestion on the behavioural and physiological responses of the fish. The results of this study will be important to evaluate the threat of microplastics to juveniles of important commercial and ecological fish species.

Keywords: Microplastic ingestion, planktivorous fish, gustatory trap, microbiota, behavioural and physiological responses

\*Speaker

## Novel methods for the determination of microplastic in sediment and biota from the Norwegian continental shelf

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NGI has developed a novel method for the extraction of microplastic in sediments and biota for further qualitative and quantitative determination by FTIR microscopy. The method involves separation in special Bauta separation towers using high density liquid and purification by organic matter removal using oxidation agents including hydrogen peroxide prior to FTIR analysis.

Keywords: Analytical method, sediment, biota, Norwegian continental shelf

\*Speaker

## Gastropod pedal mucus facilitates uptake of microplastics by marine periwinkles

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A growing number of studies is showing that ingested microplastics can induce deleterious effects in aquatic organisms. Accordingly, it is essential to identify mechanisms that facilitate the uptake of microplastics to identify crucial exposure pathways. Marine gastropods produce pedal mucus for locomotion. In order to compensate for the considerable amount of energy spent for mucus production many gastropods forage on the mucus trails of conspecifics thereby taking up particulate matter, such as microalgae, which adhere to the sticky surface of the pedal mucus. We investigated (1) if suspended microplastics adhere to the pedal mucus of the marine periwinkle *Littorina littorea* and (2) if the periwinkles ingest adherent microplastics when foraging on contaminated pedal mucus. In laboratory experiments microplastics clearly accumulated in gastropod pedal mucus. The number of microplastics in the mucus increased with the particle concentration in the seawater but was independent of the incubation time. However, older mucus retained less microplastic particles than fresh mucus indicating progressive aging of the mucus. Periwinkles readily consumed pedal mucus contaminated with microplastics resulting in the deposition of ingested microplastics in fecal material. Our results showed that the pedal mucus of marine periwinkles collects suspended microplastics from the water column and makes it available for ingestion by marine benthic invertebrates.

Keywords: ingestion, pathways, consumers, marine, mesoherbivores

\*Speaker

## Are sea squirts playing a role in the transportation of marine plastics?

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Microplastics are now found in all areas of our oceans, with many buoyant polymers being found in sediments. However, the mechanisms of this transportation of plastic from the surface to the seabed are not yet known. Marine biota may be pivotal in this transportation; ingestion and

subsequent egestion may alter the properties of plastics causing them to behave differently in the oceans. One organism that may be playing a role in this biotransformation of plastics are sea squirts. Sea squirts are prolific filter feeders capable of filtering a volume of sea water equivalent to their body volume every second. This filtering process is important for local ecosystems as sea squirts remove and sequester toxins from the water they filter. However, this high filtration rate enhances the chance of plastic ingestion by sea squirts. We looked at microplastic ingestion in the solitary sea squirt, *Ciona intestinalis*, through both laboratory and field studies. We found that *C. intestinalis* readily ingested a variety of plastics in laboratory exposures, but were able to rapidly eject them. Ingestion did not appear to significantly affect the heart rate or feeding rate of the sea squirt. However, excretion of these particles in faecal matter may be providing a mechanism for buoyant plastics to be transported to the benthos. Wild specimens of *C. intestinalis* were also analysed for the presence of microplastics. A number of plastic-like particles were found to be present within these organisms, suggesting that ascidians may be susceptible to microplastic ingestion in the marine environment.

Keywords: Invertebrates, microplastic ingestion

\*Speaker

## Microalgae as Microplastics Biofloculators

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Human consumerism and pollution gave rise to aquatic environments filled with plastic litter, susceptible to breakage and release of increasingly smaller sized low-molecular-weight polymer fragments, with increasing accumulation in the marine and freshwater environments. Microplastics (0.1  $\mu\text{m}$  - 0.5 mm) have a strong influence in the aquatic ecosystems, as they are ingested and transferred through the planktonic web and higher trophic levels, modify genetic expression and exhibit toxicity and mortality in marine plankton. Plastic particles have been found throughout oceans and rivers, from the surface to sediment on the seabed. Microalgae can excrete exopolymers (a gel-like structure) with potential to form hetero-aggregates with plastic particles. In this work two marine (*Tetraselmis* sp. and *Gloeocapsa* sp.) and two freshwater (*Scenedesmus* sp. and *Microcystis panniformis*) microalgae were exposed to microplastics. In the present work, the interactions between the microalgae and two types of microplastics (high and low density) were

characterized and differentiated in terms of the potential production of exopolymers and hetero-aggregation as a function of plastic particles type, size and density by means of fluorescence microscopy and scanning electron microscope. Results showed that the hereto-aggregates were composed by microalgae, exopolymers and microplastics and that the size, strength, viscosity and exopolymers production, were very species-specific. Thus, *Microcystis panniformis* and *Scenedesmus* sp. exhibited a considerable amount of exopolymer production, but with lesser capabilities to aggregate microplastics. *Tetraselmis* sp. displayed an excellent ability to aggregate both low and high-density beads, being only semi-limited by the size of the MP. *Gloeocapsa* sp. is a novel microalgae with outstanding exopolymer production and microplastic aggregation capabilities. The results highlight the potential of the exopolymers to interact with plastic particles and the hetero-aggregates importance for the plastics vertical transport from the water surface to the sediment.

Keywords: Microalgae, Exopolymers, Microplastics, Polystyrene (PS), Acrylic (PMMA), Hetero, aggregates

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## Does heat stress increase the susceptibility of marine bivalves to microplastic pollution?

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Previous studies already documented that the pollution of the water column with microplastic particles can have negative effects on marine filter feeders such as bivalves. However, it is still unclear whether further environmental stressors such as heat, oxygen deficiency or fluctuations in salinity, which are common in coastal habitats and should therefore frequently co-occur with microplastics, can amplify its negative effects. We investigated this concept in identical laboratory experiments that were conducted simultaneously at 4 sites in Chile, Indonesia, Japan and Wales (UK). There, we exposed specimens of 6 bivalve species, *Choromytilus chorus* and *Mytilus chilensis* (in Chile), *M. trossulus* and *Crassostrea gigas* (in Japan), *Perna viridis* (in Indonesia) and *M. edulis* as well as *C. gigas* (in Wales) to 4 concentrations of irregularly shaped PVC particles (2 – 70 µm). The microplastic concentrations, i.e. 0, 2, 20, 200 mg/l, were fully crossed with three temperature regimes of which two simulated either moderate or severe heat stress. The third regime represented the long-term average sea surface temperature at site. To assess the effects of these treatment combinations on mussel health, we measured oxygen consumption, filtrations rates and

faeces production after 12 weeks of exposure. We found significant interactive effects between heat stress and particle concentrations, which indicate that additional environmental stressors can increase bivalve susceptibility to microplastics. These interactions, however, were found in some but not in all of the test species as well as for some but not for all of the assessed response variables. We provide an overview over our results, interpret the observed interactions and discuss which factors could influence the interplay between microplastic pollution and environmental stress.

Keywords: heat stress, exposure to microplastics, PVC, bivalves, filtration, respiration, experiments, interactive effects

\*Speaker



## **November 20th – Panel 8.2: 15h45 - 17h30 (EC), Panel chaired by Melanie Bergmann.**

### **The role of vertical mixing on the global distribution of near-surface microplastic**

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Buoyant microplastic particles at the ocean surface can be submerged to deeper layers through wind induced turbulent mixing (Kukulka et al. 2012). Yet the fact that particles in deeper layers are transported by currents that are different from those at the surface has not been explored so far. We simulate the global-scale transport of passive microplastic particles constrained to different depths in the ocean from the surface to 130m depth. We compute trajectories of approx. 1 million virtual particles with the Parcels framework using hydrodynamic data from the global OFES model on 1/10 degree resolution. We find that particles constrained to deeper layers show significantly different long-term concentration patterns to those at the surface (see Figure). We also study the effect of vertical mixing on the global transport of buoyant microplastic by explicitly taking into account the vertical displacement through surface mixing in the trajectory computation according to the parameterization of Kukulka et al. 2012. We simulate both long- term distributions (garbage patches) and short-time transients through global uniform and point location release experiments. The results indicate that the locations of the garbage patches are only weakly affected by vertical mixing because deeper currents have smaller convergence. Nevertheless, deeply submerged particles follow a completely different dynamics, not governed by the surface garbage patches. These results could be relevant for understanding how microplastic can be transported to remote regions outside the garbage patches such as the Southern Ocean.

Keywords: Modelling, Lagrangian particle tracking, global microplastic transport

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# How do different physical processes affect the pathways of floating microplastic? The Parcels Lagrangian Ocean analysis framework

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The transport of plastic on continental shelves is complex. The difficulties of modelling plastic dispersion are due to the limited understanding of how it moves in the environment. Furthermore, its fate is affected by the wide range of hydrodynamics time scales and processes.

Here, we simulate the transport of floating microplastic released in the main estuaries of the European Northwestern continental shelf. Different sets of hydrodynamics data, such as NEMO, GlobCurrent and CMEMS are compared, assessing the plastic distribution sensitivity resulting from these flow fields. The connectivity between the North Sea and Arctic basins is studied in terms of microplastic transport.

To run the different simulations, we develop and use the Lagrangian ocean analysis framework Parcels, which is freely available and open source ([www.oceanparcels.org](http://www.oceanparcels.org)). The model is designed to use the output of state-of-the-art ocean models for advecting and dispersing virtual particles. To combine user-friendliness with efficiency, Parcels is implemented in Python before being just-in-time compiled in C and executed as a library. It aims to decouple the model defined by the user from the low-level implementation.

The large modularity of Parcels enables to read velocity fields from different ocean models, since it processes the discretisation of these fields naturally. Furthermore, it gives the possibility to freely define the particle dynamics, to evaluate the importance of different physical processes, such as tides, wind and waves, that drive the plastic transport.

This presentation focuses on both the dispersion of the North Sea microplastic and the details of the Parcels framework, which can be used to simulate the dynamics of very different types of particles, from floating or non-floating marine litter, to plankton or tuna fish.

Keywords: Parcels, microplastic, Lagrangian, modelling

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# A large scale mesocosm approach to simulate chemical and biological effects of microplastics in a high-plastic ocean

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The Mediterranean Sea is a closed marine environment surrounded by a complex coastal area and a population of more than 400 million. As in all populated areas, it has an elevated concentration of marine litter and microplastics, with long-term consequences that are poorly understood. The identification of accumulation hotspots, sources and fate of marine microplastics and distribution processes is a priority research area. There is rising concern that microplastics interfere with the marine carbon pump, by acting as substrates for microbial processing of organic material.

Studying such dynamics would require hypotheses testing under controlled, repeatable conditions simulating real oceanic environments. This is difficult to achieve in the field or in the laboratory. We used large-scale mesocosms to study the effects of microplastics at the ecosystem level, building on recent methodologies that have been applied to address other ocean processes and changes. We simulated a "high-plastic" ocean with high concentrations of polystyrene microspheres and in situ Mediterranean seawater in 3 m<sup>3</sup> mesocosms.

We followed the dynamics of biological and chemical characteristics of each mesocosm over two weeks, observing variations in the production of organic material in the presence of microplastics with respect to controls. We encourage collaborative projects to expand the use of these valuable infrastructures to improve our understanding of microplastics fate and effects in aquatic environments. Given their ubiquitous nature, we expect that there are numerous unrecognized feedbacks between microplastic pollution and other anthropogenic-driven ocean changes.

Keywords: Microplastics mesocosms ocean simulation

\*Speaker

# MODELING PLASTIC WASTE FLOWS IN THE MEDITERRANEAN ENVIRONMENT: A SOURCE TO SINK APPROACH

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The Mediterranean Sea is one of the most polluted zones of the world with more than a million debris of plastic floating per km<sup>2</sup>. While garbage patches have been observed in the oceans, there is still no accumulation zone clearly identified in the Mediterranean Sea. In order to better understand the impact of plastic pollution on marine ecosystems and potentially on human health, it becomes necessary to better quantify the plastics sources and to analyze their dispersion offshore. Here we present a modeling approach that will combine statistical models of plastic inputs from rivers and other land-based sources into the sea with a numerical model of ocean currents. Quantification of plastic wastes that enter the Sea annually is based on the delineation of Mediterranean watersheds and geo-referenced data for potential drivers such as population densities, waste management and river drainage basin characteristics. It is this part of our work that will be presented during the conference. In a further step, the model of plastic inputs will be used to initiate numerical simulations of ocean circulation to analyze the plastics dispersion through the currents on the basis of the 3D hydrodynamic model SYMPHONIE. This model commonly used for sediment transport is able to integrate plastic particles with different size and density. Our modeling approach will bring knowledge both on the origin and fate (beaching, sedimentation on the seabed, cascading or surface drift) of plastics in the Mediterranean environment based on realistic particle and mass concentrations. This will enable to integrate the increasing number of field observations and measurements into realistic scenarios on plastic pollution in this key environment for global change.

Keywords: continent, ocean transfer, plastic pollution, Mediterranean Sea, ocean circulation modeling

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# Numerical Model Simulation to improve the understanding of Micro-Plastics Debris in marine environments: sensitivity of microplastics fate to particles physical properties and behaviour

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Understanding Marine micro-Plastic Debris (MPD) movement is a global issue of international concern. Physical properties and processes of MPD particles can determine their motion and fate in ocean and coastal systems. However, most of models of MPD movement assumed MPDs as neutral particles drifting within the surface layer. These models are typically coupled to a specific ocean model and focus on large spatial scales. In this study, we present TrackMPD, a new non-Lagrangian tracking model for marine debris transport that consider (1) the behaviour of the particles of different densities, sizes, shapes and fouling states, and (2) physical processes such as windage, dispersion, degradation and refloating. TrackMPD is compatible with velocity data from many different hydrodynamic models (e.g. POM, ROMS, MITgcm and FVCOM) and can compute forward and backward trajectories in two or three dimensions. We describe and quantify the influence of different microplastics behaviours and physical processes on the trajectory and fate of microplastics using Jervis Bay (SE Australia) and its adjacent coast as natural laboratory. Preliminary results show that the microplastics dynamical properties impacting their sinking have a large effect on the distribution of MPDs, even more than other major physical processes such as dispersion and refloating. In particular, the plastic density (Fig 1) and the fouling state play a key role on the transport and final fate of both spherical and cylindrical microplastics, while the particle size only has a high impact on the movement of spherical particles. A detailed sensitivity analysis ranks the relative influence of each model parameter on the distribution and fate of MPDs. These findings highlight the importance of the three-dimensional tracking of microplastics, and can help prioritise experimental research on the particle dynamical properties and physical processes involved on plastic transport, leading to more accurate predictions of their fate.

Keywords: microplastics transport, 3D modelling, dynamical properties, physical processes, mi-

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# Laboratory experiments and numerical modelling of plastic particles beaching

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The ubiquitous presence of plastic debris in the world's oceans is globally recognized as a key environmental challenge. The large amount of debris drifting in the ocean constitutes a grave threat to marine life and ecosystems. Tackling this environmental challenge requires an understanding of how much plastic is in the ocean, where it is accumulating and how it is transported there. However, the fate of a large proportion of plastic currently thought to be in the ocean is still unknown due to the difficulty of obtaining representative samples of plastic debris and the presence of sinks, which include shorelines (beaching), the ocean bed and ingestion by marine life. Within the Tracking Of Plastic In Our Seas (TOPIOS) project, laboratory experiments and numerical techniques are combined to study the plastic fluxes through the coastline and the plastic returning to the emerged beach (beaching) as a function of wave climate and plastic properties. Wave flume experiments to quantitatively determine those fluxes are performed in controlled conditions varying the wave steepness, and energy; and plastic particles density and size. A plastic particle tracking system using video cameras and video image analysis is used. In parallel a numerical model is being developed combining a hydrodynamic solver, SWASH, and a Lagrangian tracking model, Parcels. The Lagrangian tracking solver is modified to introduce the particle inertia, drag, buoyancy, added mass and turbulent dispersion. Particle beaching is estimated using the advection length, defined as the maximum distance seawards from the shoreline from which particles end up beaching. The attached figure shows laboratory experiments configuration (left), measurements of 3D lagrangian trajectories (middle) and numerical and experimental advection ratios (right). The full paper will present details of the experimental technique, experimental data analysis of the plastic particles beaching and numerical modelling results.

Keywords: plastic beaching, laboratory experiments, coastline, plastic transport

\*Speaker

# Why does floating microplastic accumulate in the subtropical gyre of the North Atlantic Ocean?

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While floating microplastic has been found in most of the world's oceans, it is distributed unevenly over the globe, with elevated concentrations in the subtropical ocean gyres in each ocean basin. However, little attention has been given to the different physical mechanisms responsible for this accumulation, with previous studies either focusing solely on the Pacific Ocean or simply attributing the accumulation to wind-driven Ekman current convergence. In our research, we have studied the roles of various current components such as the wind-driven Ekman currents, pressure gradient-driven geostrophic currents and surface wave-driven Stokes currents in determining the location of microplastic accumulation regions in the North Atlantic. We have run Lagrangian particle simulations of floating microplastic to locate the position of greatest accumulation. Hydrodynamic flow fields from the GlobCurrent and WaveWatchIII re-analysis projects were obtained for the Ekman, geostrophic, total (Ekman + geostrophic) and Stokes currents, thus allowing the use of assimilated rather than modelled circulation. By observing the final microplastic distribution with each of the different current components, the respective contributions to the microplastic accumulation could be isolated.

The model runs using the total currents resulted in a peak microplastic accumulation near the middle of the North Atlantic in the subtropics (Figure 1), which matches observations and the results of earlier modelling studies. The location of this peak accumulation is due to the wind-driven Ekman currents, with the geostrophic currents not leading to accumulation in the subtropics. The Stokes currents act as an accumulation mechanism in the subtropics, but also lead to microplastic transport towards the equatorial and polar regions. This improved understanding of the contributing mechanisms behind the accumulation of microplastics can benefit a range of follow-up studies, including the efficacy of open-ocean plastic clean-up efforts.

Keywords: Global Ocean Circulation, Lagrangian Modeling, Stokes Currents, Garbage Patch, Microplastic Accumulation

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# Tracking nanoplastics in aquatic organisms at environmentally realistic concentrations

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Based on discharge models (1), at least 12 % of plastic litter that should be accumulating in oceans is missing – or not detectable with actual analytical means. The fragmentation of plastic debris into smaller particles, such as the largely "undetectable" nanoplastics (NP,  $d < 1000\text{nm}$ ) has been suggested to be a potential explanation for this missing sink in the environment. Due to concerns that the increasing production of manufactured and possibly non-manufactured NP might present unintended hazards, a small number of studies have also investigated the uptake or the effects of NP into biota. However, they all used concentrations exceeding those predicted to occur in the environment; the latter range between  $1 \text{ pg L}^{-1}$  and  $15 \text{ } \mu\text{g L}^{-1}$ (2). In fact, most studies used concentrations between two and seven orders-of-magnitude higher than those predicted. For instance, some studies attempted to track fluorescent particles, using surface-functionalized polystyrene nanoparticles, to produce qualitative characterization of the ingestion of MP/NP and tissue distributions in transparent aquatic organisms. However, the use of such methods has important constraints, including high limits of detection, interference from fluorescent backgrounds, the range of organisms that can be used and the weak resolution (e.g. due to internal light diffraction/reflection) that can be reached. Overall, the important challenges that tracking plastic particles in biological or environmental media represent, have impeded the quantification of the uptake and the characterization of the accumulation patterns in realistic environmental settings. In this presentation, we report the first biokinetic and quantitative tissue distribution data produced in a study where aquatic organisms (i.e. the scallop *Pecten maximus*) were exposed to predicted environmentally realistic concentrations ( $15 \text{ } \mu\text{g L}^{-1}$ ) of two sizes of NP ( $d = 20$  and  $250 \text{ nm}$ ). (1) Cozar, et al. (2014) Proc Natl Acad Sci 111(28):10239–10244. (2) Lenz, et al (2016) Proc Natl Acad Sci 113(29):E4121–E4122.

Keywords: biokinetics, isotopically labelled, tissue distribution, nanoparticles

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## November 20th, JPI Oceans panels, Microplastics Projects Joint Final Meeting (AGH).

**9h-9h10**, Welcoming Remarks, Objectives of the Day. Tim Eder, Federal Ministry of Education and Research, Germany.

**9h10-9h55**, Project results BASEMAN: (i) General Introduction (Gunnar Gerdts); (ii) From the cradle to the spectrum (Jesus Gago); (iii) Analytics of microplastics (Sebastian Primpke) and (iv) Challenges, limitations and opportunities in identifying small microlitter particles (Martin Hasselöv).

### **Defining the baselines and standards for microplastics analyses in European waters!?** **Highlights and pitfalls of JPI-O BASEMAN**

*Gunnar Gerdts (Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Helgoland, Germany) et al (the BASEMAN consortium)*

Since the middle of last century rapidly increasing global production of plastics has been accompanied by an accumulation of plastic litter in the marine environment. Dispersal by currents and winds does not diminish the persistence of plastic items which degrade and become fragmented over time. Together with micro-sized primary plastic litter from consumer products these degraded secondary micro-fragments lead to an increasing amount of small plastic particles (smaller than 5 mm), so called “microplastics”. The ubiquitous presence and massive accumulation of microplastics in marine habitats and the uptake of microplastics by various marine biota is now well recognized by scientists and authorities worldwide. A fundamental issue precluding assessment of the environmental risks arising from microplastics is the lack of standard operation protocols (SOP) for microplastics sampling and detection. Comparability of data on microplastics concentrations was (and still is) hampered by the huge variety of different methods applied, generating data of extremely different quality and resolution. JPI-O BASEMAN as one of four projects funded in the framework of the JPI-O pilot action “Ecological Aspects of Microplastics” aimed to overcome this problem by profound and detailed comparison and evaluation of all approaches from sampling to identification of microplastics in the marine environment. However, due to the increasing perception of microplastics as emerging pollutant(s) in the environment (not only marine), these approaches now need to be harmonized with those developed (and applied) in freshwater and terrestrial systems for a holistic understanding of the MP-pollution in interconnected ecosystems. In the framework of the JPI-O Microplastics Projects Joint Final Meeting we will present our findings and methodological suggestions concerning sampling and detection of MP in the marine environment („From the cradle to the spectrum“, Jesus Gago) and our progress related to analytical approaches („Analytics of microplastics“, Sebastian Primpke & Barbara Scholz-Böttcher; "Challenges, limitations and opportunities in identifying small microlitter particles", Martin Hasselöv).

**9h55-10h40**, Project results WEATHER-MIC: (i) General introduction (Annika Jahnke); (ii) The influence of weathering on the sinking behavior of microplastic (Erik Toorman); (iii) Characterization and effect testing of leachates liberated during UV light-induced weathering of polymers (Christoph Rummel) and (iv) Ecological relevance of microplastic effect testing with a focus on weathering (Zandra Gerdes).

### **WEATHER-MIC – How microplastic weathering changes its transport, fate and toxicity in the marine environment**

*Annika Jahnke<sup>1</sup>, Christoph Rummel<sup>1</sup>, Matthew MacLeod<sup>2</sup>, Annegret Potthoff<sup>3</sup>, Elena Gorokhova<sup>2</sup>, Zandra Gerdes<sup>2</sup>, Erik Toorman<sup>4</sup>, Mechthild Schmitt-Jansen<sup>1</sup>, Dana Kühnel<sup>1</sup> and Hans Peter H. Arp<sup>5</sup>*

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Studies on plastic debris including microplastic in the aquatic environment are increasing. However, it is still common to use pristine particles, which are of limited relevance since plastic debris is weathered quickly when exposed to various environmental factors. The overall aim of the JPI Oceans-funded project WEATHER-MIC was to assess the impacts that weathering has on the transport, fate and effects of plastic debris and the related leachates, i.e., the chemicals being liberated during weathering.

There is a range of environmental factors influencing the weathering of plastic material and leaching of chemicals, including UV radiation, physical stress due to wave action near- and onshore, and microbial degradation. Their relative importance depends on site-specific factors and the polymer. As additives are not chemically bound in the polymers, they are expected to leach relatively fast. Microbial colonization and biofilm formation on the polymer surface has several implications: Besides shielding the particle from UV light, the polymer's density is altered, resulting in changes in buoyancy and sedimentation rate. Furthermore, biofilms add an additional sorptive phase to the plastic fragments, which can slow down the sorption of chemicals and leaching from the polymer. Furthermore, the fragmentation of large plastic debris leads to formation of smaller, irregularly shaped particles with relatively large contributions of biofilm per particle mass. These particles can be ingested by marine consumers and hence potentially affect organism fitness as well as facilitate transfer in food webs.

In this overview, we present WEATHER-MIC results, with a focus on three main topics: (i) The influence of weathering on the sinking behavior of microplastic; (ii) Characterization and effect testing of leachates liberated during weathering of polymers induced by UV exposure; (iii) Ecological relevance of microplastic effect testing with a focus on weathering. Additional important aspects, such as advanced particle characterization, will also be addressed.

10h40-11h, Coffee Break.

**11h-11h45**, Project results PLASTOX: (i) General Introduction (Andy Booth); (ii) Uptake and Accumulation of microplastic (Laura Airoidi); (iii) Toxic effects of ingested microplastic (Paula Sobral); (iv) Adsorption and desorption of pollutants to microplastic (Kaori Sakaguchi-Soder) and (v) Bioavailability of pollutants adsorbed to microplastic (Andy Booth).

### **"PLASTOX: A big assessment of small particles?"**

*Andy Booth<sup>1</sup> and the PLASTOX consortium for JPI Oceans.*

<sup>1</sup> SINTEF Ocean – Postboks 4762 Torgard, N-7465 Trondheim, Norway

Although MP ingestion has been demonstrated for a range of species representing most trophic levels, the number of studies reporting impacts associated with MP ingestion is small. Impacts observed for different species include reduced feeding, weight loss, reproductive and developmental abnormalities, and phagocytic activity and inflammatory responses. These impacts are significantly influenced by particle size, with smaller sizes typically eliciting more significant effects on organisms. The role of MPs as vectors for transporting known environmental pollutants (e.g. persistent organic pollutants and metals) appears to be important. However, there is currently insufficient data available to be able to draw strong conclusions regarding the impacts of MPs on marine organisms. Furthermore, there is a lack understanding regarding the effects of MP exposure and uptake at the population level and across food webs. There remains a need to address the following knowledge gaps concerning MP effects:

- Potential effects of MPs on marine species at different life stages
- Determination of MP uptake, internalisation and potential for trophic transfer
- Understanding the role of MPs as vectors for exposure and bioaccumulation of POPs and metals
- The role of plastic additive chemicals on the potential effects of MPs to aquatic species
- Use of environmentally relevant MP test materials and the importance of physicochemical properties

PLASTOX is funded under the JPI Oceans Pilot Action “Ecological Aspects of Microplastics”. The project investigates the ingestion, accumulation, food web transfer, and ecotoxicological impact of MPs, together with persistent organic pollutants (POPs), metals and plastic additive chemicals, on key European marine species and ecosystems. Acute and sublethal ecotoxicological effects of MPs are assessed on marine organisms from phyto- and zooplankton to shellfish and fish. Adsorption and desorption behaviour of organic and inorganic pollutants to MPs are investigated using common POP and metal contaminants. The influence of MP physicochemical properties (size, shape, surface area and composition) on these processes will be evaluated. To study ecological effects of MPs, laboratory tests and mesocosm studies will be combined with field-based observations and manipulative field experiments at stations representing a wide range of European marine environments (Mediterranean, Adriatic, North, and Baltic Seas and the Atlantic).

**11h45-12h30**, Project results EPHEMARE: (i) General Introduction (Ricardo Beiras); (ii) Uptake and accumulation (Ronny Blust); (iii) Trophic Transfer (Thomas Braunbeck); (iv) Toxicity assessment (J Bellas) and (v) Field validation (Stefania Gorbi).

## **ECOTOXICOLOGICAL EFFECTS OF MICROPLASTICS IN MARINE ECOSYSTEMS (EPHEMARE); SYNTHESIS OF RESULTS**

R. Beiras\*, S. Keiter, R. Blust, X. Cousin, M. Albentosa, T. Braunbeck, F. Regoli, K. Kopke

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Microplastics (MP) have been found in every marine compartment where they have been searched, from surface water to the bottom sediments, and in most marine species. Microplastics, initially defined as plastics below 5 mm, have been traditionally sorted out by eye or under binocular microscope. Manta trawls, neuston and plankton nets do not catch particles smaller than ca. 300 µm, while those taken up by marine organisms are typically smaller than 100 µm. Different organisms can be used as bioindicator species for MP depending on their habitat or diet preference, mussels being particularly suitable for fibers. The most frequent typologies of microplastics extracted in marine biota were PE (61%) followed by PP (19%).

MP have been shown to be easily ingested by zooplankton and transferred across these food webs, but they are not acutely toxic (LOEC>30 mg/L) in the standard 24-48 h acute tests. New, promising endpoints related to behavior, immune response, reproduction and development seem more sensitive to test microplastic toxicity. Jellyfish larva behavior is affected at environmentally relevant levels of MPs. Fish exposed to MPs are susceptible to stress increasing mortality rates, inducing apoptosis, immunosuppression and histopathological damage. Long-term dietary exposure lasting from hatching until adulthood reduced female growth, decreased reproductive output, and increased abnormal offspring of embryos and larvae. Multi-stress experimental setups seem most adequate to test the potential effects of MPs. MPs are not necessarily the prime cause of toxicity but aquatic organisms may become more sensitive to additional insults in their presence. The novel ecotoxicological tools developed within the framework of EPHEMARE may contribute to set more protective environmental quality standards for microplastics than current standard tests that fail to detect certain effects and interactions.

MP from consumer products obtained after compounding with chemical additives are more toxic than polymer resins for both PE and PVC. Concerning chronic effects some plastic additives have shown reproductive toxicity due to their endocrine disrupting properties, and have been restricted in food-contact, children products or medical applications. However, even in those cases the information of the composition of plastic products is not disclosed to the consumers. In fact, no information at all on additives is included in labeling of consumer products which makes very difficult to conduct a proper hazard or risk assessment.

**12h30-13h15**, Lunch including Poster Session.

**13h15-14h**, Cross-cutting theme I: Towards standardized Methods. *Short presentations (max. 6-7 min) followed by an open discussion of the topic, chaired by Gunnar Gerdts*: (i) Standardised methodologies: Challenges of implementation (Joao Frias); (ii) Quantifying trophic transfer (Laura Airoidi); (iii) Measuring presence and effects of MPs in biota (Francesco Regoli); (iv) The cost/information issue in microplastics research (Arne Petersen) and (v) Towards a standards Initiative (Andrea Held).

**14h-14h45**, Cross-cutting theme II: Weathering and Degradation. *Short presentations (max. 6-7 min) followed by an open discussion of the topic, chaired by Annika Jahnke*: (i) Annegret Potthoff: Advanced particle characterization and weathering-induced changes (WEATHER-MIC); (ii) Soledad Muniategui-Lorenzo: Highlights of the Photodegradation of the Baseman's polymers (BASEMAN); (iii) Ketil Hylland: The relevance of microplastic weathering for environmental impacts (EPHEMARE) and (iv) Andy Booth: UV, mechanical and hydrolytic degradation of synthetic polymer fibres from textiles (PLASTOX).

**14h45-15h**, Coffee Break.

**15h-15h45**, Cross-cutting theme III: Ecotoxicological Impacts on Marine Organisms. *Short presentations (max. 6-7 min) followed by an open discussion of the topic, chaired by Ricardo Beiras*: (i) Biofilm - Plastic interactions (Mechthild Schmitt-Jansen, WEATHER-MIC); (ii) Title TBD (BASEMAN); (iii) Uptake, transfer and toxicity of microplastics - from unicellular organisms to vertebrates (Xavier Cousin, EPHEMARE ) and (iv) Effects of ingested and accumulated microplastics (Paula Sobral, PLASTOX).

**15h45-16h30**, Cross-cutting theme IV: Adsorption and Additives. *Short presentations (max. 6-7 min) followed by an open discussion of the topic, chaired by Andy Booth*: (i) Sorption of persistent organic pollutants on microplastics in European marine environment (Kaori Sakaguchi-Soder, PLASTOX); (ii) Sorption and bioavailability of model pollutants on microplastics (Steffen Keiter, EPHEMARE); (iii) PLASTOX and BASEMAN; lessons learned from the perspective of pollutants hitch hiking with plastic particles (Dorte Herzke, BASEMAN/PLASTOX) and (iv) Release of chemicals from plastic under strong UV radiation (Merle Plassmann, WEATHER-MIC).

**16h30-17h**, Concluding Remarks

**18h-19h15**, Science for Policy: Marine Strategy Framework Directive - Technical Group Marine Litter Position Paper, Georg Hanke, JRC. Panel Discussion :Andy Booth, Francesco Regoli, Annika Jahnke, Gunnar Gerdts and TG Marine Litter Representatives. Moderation: N.N., TG Marine Litter.

**19h15-19h30**, Presentation 2<sup>nd</sup> JPI Oceans joint call on microplastics. Tim Eder, Federal Ministry of Education and Research, Germany.

**19h30**, Cocktail.

## November 21st

### November 21st – Panel 10.1: 8h30 - 10h (AGH), Panel chaired by Paula Sobral.

#### Microplastic distribution in coastal waters

Bhavani Narayanaswamy \*† 1, Solene Girardeua-Potel 2, Callum Whyte 1

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Microplastics (MPs) are particles smaller than 5mm in length/diameter. They have been found in every marine habitat that has been sampled for microplastics. Although MPs have been studied in coastal regions, regular sampling of the habitat has not been undertaken, leading to a lack of knowledge as to how MP abundance may vary over time. Water samples were collected from four sites around Scotland, two mainland and two island locations over a 9-month period. Three of the sites were on the westward facing side of the mainland/island. Samples were collected using a Lund tube from a depth of 8m. After collection samples were stored in the fridge until processing could take place. Water sample aliquots were allowed to settle in Hydrobios settling chambers following the Utermöhl method for 20 hours. The chambers were then analysed with total MP counts being undertaken.

There was considerable variation in the number of microplastic particles present in the water samples when comparing location and the time-frame. Maximum number of MPs collected in a sample varied from > 5000 MPs/l at a west coast island station, to a low of ~ 30 MPs/l at an east coast mainland site with a mean of 1230 MPs/l, through to 44 MPs/l at these same sites.

These preliminary results indicate big differences between locations i.e. west coast stations vs east coast stations, but smaller differences between mainland and island sites. The hydrography of the region undoubtedly influences the presence of MPs present in the water column. Particle modelling in this area, found that cells in the surface waters of the west coast region would be influenced by the Slope Current originating from the Atlantic and the Scottish Coastal Current coming from the Irish Sea. Further analysis on the data especially in relation to other activities will be undertaken.

Keywords: Microplastics, coastal waters, Scotland, hydrography

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## Spatio-temporal monitoring of coastal marine plastics

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Coastal ecosystems are continuously affected by anthropogenic impacts such as urbanization, maritime activities, recreational and commercial activities, all of which have been prominent sources of plastic marine litter. Plastics are continually entering the marine environment and overtime degrade posing a potential threat to marine wildlife. Currently in the Mediterranean Sea, the concentration of plastic particles is between 0.116 – 0.40 items/m<sup>2</sup> weighing an estimated 671.91-2020 g/km<sup>2</sup>. The current study aims to quantify and identify the spatial distribution of marine plastics over time in coastal ecosystems of the Island of Mallorca in the archipelago of the Balearic Islands located in the Western Mediterranean Sea. Sea surface samples were collected during summer months in 2017 (July, August and September) at seven locations across the island within 500 meters of the coastline. Three samples at each location were collected for posterior quantification of floating plastics at the laboratory to assess autocorrelation within sampling locations. Plastic items was observed in all collected samples of varying sizes, ranging from macro- (> 25 mm), meso- (5-25 mm) and micro-plastics (< 5 mm), composed principally of fragments, films and filaments. Despite initial results show plastics were present in the sea surface at all sampling locations surrounding the island, no significant differences were seen between locations (KW,  $p > 0.05$ ). These preliminary results indicate the coastal marine plastic concentrations are similar to those found offshore in the Western Mediterranean Sea.

*Keywords: microplastics, marine debris, neuston nets, coastal*

*\*Speaker*

## From coastal to oceanic micro-meso and macroplastics in the SE Bay of Biscay

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Several numerical models predict that the SE Bay of Biscay is a critical area for oceanic litter accumulation because of its geography and metocean conditions; and yet, the knowledge of marine litter is limited in the region. This has led local authorities to seek scientific advice to manage this new challenge. Here we show the results of 3 oceanographic surveys that cover different oceanic regions of the Bay of Biscay: BIOMAN (May 2017), ETOILE (August 2017) and LEMA (Sept-Nov 2017 and May-Sept 2018), where 210 water surface samples were taken with a 500  $\mu\text{m}$  mesh-size neuston net. Results map the spatio-temporal distribution of micro-mesoplastic abundance, from coastal to oceanic waters. Micro-mesoplastic abundance in general follows an expected gradient from coastal to oceanic waters, but also shows high variability within the same water mass. Some coastal water samples contained high abundance levels with almost  $1500 \cdot 10^3$  items  $\text{km}^{-2}$  in coastal waters in contrast to low levels ( $16 \cdot 10^3$  items  $\text{km}^{-2}$ ) in deeper waters, while no plastic was found in others. Such variability highlights the importance of understanding how the meso and small-scale ocean features (e.g. eddies, fronts), which are frequent on coastal waters, may influence and help to predict micro-mesoplastic accumulation hotspots for management purposes. Micro-mesoplastic abundance and characterisation results are completed with macro-litter mapping obtained for the same region by: (i) fishing vessels devoted to collect marine litter from coastal waters (May-September 2018); and (ii) visual detection of macro-litter (JUVENA and BIOMAN ecosystemic surveys). We anticipate that our results have started to provide data of marine litter abundance and sources, and that they are currently being used as a baseline to work with relevant authorities, sectors and industries (such as the fishing sector) regarding their responsibility in the prevention and the development of management strategies to deal with marine litter locally.

Keywords: Microplastic, Mesoplastics, Macroplastic, SE Bay of Biscay, management strategies,

\*Speaker

## Microplastics in the Sea Surface Microlayer in Southampton Water, UK

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Microplastics (MP) are detected in sediments and surface waters globally. They are considered



ubiquitous pollutants, but there is little knowledge on how microplastics transfer between these two environmental compartments. One proposed pathway for transfer in estuarine and coastal environments is the sea surface microlayer. The sea surface microlayer (SML) is defined as the upper 100µm of the sea, and has been recorded as containing large abundances of microplastics. It is thought that high microbial activity in the SML produces a 'sticky' microgel, which may play a role in the trapping of microplastics in this layer. The SML is potentially a transfer pathway between the sea surface and upper intertidal sediment accumulation zones. In calm conditions, where microplastics have been able to accumulate in the SML due to a lack of turbulent mixing, the flooding tide will cover mudflat environments. This may remobilise or incorporate surface deposited microplastics into the SML, and transport these into upper intertidal environments, such as upper tidal flats and saltmarshes. This potential transfer mechanism has yet to be investigated, with limited data on microplastics abundance and behaviour in the SML. A new method for sampling the sea surface microlayer for microplastics using a dipped glass plate is detailed by Anderson et al. (2018). Glass plate sampling was found to recover more microplastics than other methods of sampling the sea surface microlayer, and also allow rapid shore-based sampling. Thus, this method was selected to determine the spatial and temporal distribution of microplastics in the SML in Southampton Water, UK, where MP abundances of up to 93 fibres/L have been observed. In particular, variations in SML MP abundance during a tidal cycle were investigated by high-frequency sampling in a saltmarsh creek, to evaluate the supply and retention of MPs in the upper intertidal zone.

Keywords: microplastics, sea surface microlayer

\*Speaker

## Distribution of microlitter in a gradient from a city to sea to identify main sources

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In order to launch effective mitigating measures for microlitter it is essential to be able to identify which compositions and characteristics of microlitter that is most abundant so links could be made to which human activities and pathways that should be targeted. Microlitter is very heterogeneous group of matter which vary not only in size, but also largely in composition, shape and density, which influence propensity to remain buoyant or deposit to the sediment close to sources. We investigate the distribution of microlitter (> 1 µm) in the surface water and in sediments

from a city (Uddevalla, Sweden) and out to the ocean (Havstensfjorden). A horizontal gradient of surface water collected with manta trawl (300  $\mu\text{m}$ ), pump (100  $\mu\text{m}$ ) and ruttner sampler (10 and 1  $\mu\text{m}$ ) and sediment samples were taken together with a vertical gradient of water samples from the deepest part in the fjord. Particles larger than 100  $\mu\text{m}$  were characterized visually and identified with FTIR whereas the smaller particles will be identified with Raman. From the surface water the main classes of identified particles were low density plastic particles, paint particles and paraffin while in the sediment the main classes of particles were high density plastic particles, combustion particles, paint particles and fibers. Highest concentration of particles was found closest to the city with a decreasing amount following the gradient out to the ocean. For the manta trawl the concentration ranged from 5.9 to 0.1 plastic particles per  $\text{m}^3$  and for the sediment samples (> 300  $\mu\text{m}$ ) between 1.9 and 0.2 particles per gram of dry sediment excluding fibers. The smaller filters (10  $\mu\text{m}$ ) follow the same trend as the larger one with higher concentration of particles closer to the city with a concentration between 700 and 346 particles/L where most particles were between 10 and 20  $\mu\text{m}$ .

Keywords: microlitter, FTIR, Raman

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## **November 21st – Panel 10.2: 8h30 - 10h (AGH), Panel chaired by Jesús Gago.**

### **Can a microbial community be artificially evolved to degrade plastic?**

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Up to 12.7 million tons of plastics are thought to enter the oceans every year, and this figure is likely to increase. As recalcitrant synthetic polymers, plastics are notoriously difficult to degrade in the marine environment and are therefore expected to persist indefinitely. To learn about the likely fate of these synthetic polymers in the ocean, we can investigate the fate of recalcitrant natural marine polymers, which are biodegraded by marine bacteria. Through artificial selection of a whole microbial community, we have obtained a microbial community that degrades the natural, recalcitrant polymer, chitin, more efficiently than that which naturally occurs. We found that the evolved community exhibited higher chitinase activity, and therefore higher potential to degrade chitin. We characterised the community using MiSeq and by obtaining isolates and found that it became enriched in organisms that are able to degrade chitin, but only when important methodological caveats, relating to the incubation time between selections, were met. We are currently applying the same method to explore the degradability of the commonly used packaging plastic poly(ethylene terephthalate) (PET). Here, the community that is best at degradation is determined based upon its ability to grow with PET as the sole carbon source. We have carried out a community succession experiment prior to the selection experiment using a range of different types of PET (i.e. PET, weathered PET, low-crystallinity PET, PET monomer) and will carry this out again at the end of the selection. The community succession here is assessed using metabolic activity assays, amplicon sequencing of the community and chemical monitoring for PET degradation products. The results of this study will inform us on the potential for microbial communities to develop the ability to degrade marine plastics.

Keywords: Degradation, Microbial community, Colonisation, Succession, Evolution, Biodegradation

\*Speaker

## A deeper look into the biodegradation of micro-plastics through metabolomics approach.

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The environmental diagnosis carried out so far on micro-plastic pollution provides information on the sources, the abundance, the distribution of plastic debris and their biotic or abiotic interactions with the environment. The interactions of polymers with microorganisms result in the formation of biofilms with complex communities and a wide range of metabolic activities. Among these microorganisms, some have the ability to degrade and metabolize synthetic polymers, through the production of enzymes or the establishment of co-metabolism. While an increasing number of studies has demonstrated bacterial degradation of plastics, the metabolic pathways involved in the biodegradation and the ultimate fate of plastic debris are still poorly understood. We developed a non-targeted metabolomics approach to assess the biodegradation processes involved in micro-plastics degradation. The study was carried out on pre-aged polyethylene fragments that provide low molecular weight compounds and support the growth of bacteria. An efficient and reproducible method was implemented to measure and compare the intracellular metabolites of *Rhodococcus rhodochrous*, an ubiquitous bacteria strain, cultivated with or without polymer. The metabolites extracted were analyzed by liquid chromatography coupled to high-resolution mass spectrometry Orbitrap. After data treatment, statistical analysis shows that the profile of metabolites produced by *R. rhodochrous* were significantly different for bacteria growing on a polymer as a sole carbon source. These results confirm the involvement of a specific metabolism in presence of plastic and allow the annotation of putative biomarkers. This study highlights the benefit of systems biology approaches to provide new insights into the metabolic functioning of microorganisms onto plastics. Such approaches could be used to investigate interaction between micro-plastics and microorganisms, determine the ultimate fate of plastics and estimate their importance in biogeochemical cycles.

Keywords: biodegradation, metabolomics, metabolites, LC/MS, Fate of micro, plastics

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# Beyond oil degradation: Enzymatic potential of Alcanivorax strain to degrade other natural and synthetic polymers

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Worldwide plastic production has been increasing year by year since its industrialization began. The macromolecular architecture of these polymers has generated materials with high performance, applicability, and durability. However, these qualities also make plastics highly recalcitrant materials, which, as a consequence, contributes to their accumulation in the environment. Due to the environmental implications of plastic pollution, it is relevant to know how these materials are potentially being degraded in the environment. In this context, bacterial isolation efforts were conducted using samples of plastic debris. From this procedure, it was possible to obtain a strain of *Alcanivorax*, a genus that has been previously linked to the degradation of contaminants in marine environments, especially alkanes from oil spills. Here, we have assessed the ability of this strain to degrade different natural and synthetic polymers, for instance, polyhydroxyalkanoates, alkanes, polyethylene (PE) and monomeric units of polyethylene terephthalate (PET). In order to understand if this strain has the potential to degrade plastics, several approaches have been taken. We have sequenced and annotated the bacterial genome, degradation experiments have been conducted using different polymers as a sole source of carbon and energy, and further cellular and exoproteomic analyses from these cultures have been carried out. Our results indicate an active process of degradation of *Alcanivorax* when using different polymers as the source of carbon; the exoproteomic profiles of cultures grown on long-chain alkanes and polyethylene indicates the up-regulation of several monooxygenases, esterases, peroxidases, and enzymes related to the metabolism of long-chain fatty acids. Enzymes similar to these have been involved in the degradation of recalcitrant polymers such as long-chain alkanes and PE.

Keywords: Biodegradation, Marine, Bacteria, Plastics, Polymers

\*Speaker

# Can biodegradable plastic contribute to the mitigation of marine plastic pollution?

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Marine plastic pollution is a growing problem, and has become a priority topic on the international agendas. Solutions as reducing, reusing and recycling matched with consumer awareness, producer responsibility and regulation are needed, as is a strong improvement of waste management worldwide. So-called bioplastics are also considered as one possible solution. However, already the term "bio" is not uniformly used, and needs precise definitions. Reliable scientific knowledge about the performance of plastic materials in the marine environment claimed to be "biodegradable" is still limited. Our results from systematic in-situ tests in several marine settings show that there are polymers and blends with this material property. The availability of plastics that are biodegradable under marine conditions within reasonable time meets the discussion whether at all, and if, where these new materials could be most efficiently be used to mitigate the perpetual pollution with plastic. Especially in sectors where (micro-)plastic loss to the environment is intrinsic to its use, like abrasion (e.g. of car tyres), or where the loss during use is highly probable (e.g. fishing gear) biodegradable materials could make a difference. We estimated the substitution potential for biodegradable polymers on the material-based, habitat-based and item-based level from data sources in the literature, and from beach waste analyses in order to support further technological development and governance. Truly biodegradable plastic could have its role in fighting marine plastic pollution. A replacement of some applications and items could be seen as a transitory measure, but the general replacement of conventional with biodegradable plastic cannot be recommended. Plastic in the natural environment is pollution - also if biodegradable. However, biodegradable plastic will not endlessly accumulate and last "forever". It also can reduce the environmental impact where loss can hardly be avoided.

Keywords: biodegradable plastic, mitigation, in situ test, substitution potential

\*Speaker

# The determination of half-life of biodegradable polymers in the marine environment

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Both bio-based and blue economies are growing. New, more sustainable materials, being for example bio-based and biodegradable, are increasingly employed. Unfortunately, we have to assume that in the near future also such new materials will end up in the environment, either by littering, loss or simply by abrasion. Therefore, data for risk assessments are needed. To avoid similar negative environmental effects as with conventional plastics, biodegradable materials shall be tested and their inherent biodegradability proven. Biodegradation is the complete conversion of the material into CO<sub>2</sub>/CH<sub>4</sub>, water and biomass. Such tests are usually done using standard test protocols. One part of the European Commission funded project Open-Bio was to develop test schemes for marine conditions. A 3-level approach was established to test new polymers in an optimized laboratory system, a semi-controlled mesocosm system, and in the field under natural coastal conditions. All materials which showed proof of inherent biodegradation in the laboratory tests did show disintegration in the mesocosm and field tests. Because a test under real field conditions indicates how long the material will persist in nature, it is mandatory to complement laboratory with field tests, ideally using the same matrices. Here, we present the determination of the half-life of a material specific for one environment. Since marine ecosystems comprise many habitats, in which conditions vary, tests appropriate for the relevant conditions are needed in order to assess the half-life in the marine environment. Our outlook presents the next steps needed in order to achieve a comprehensive environmentally relevant test scheme ready for standardisation. All data combined can fuel into a sound risk assessment of new polymers which might or will enter the marine environment, and will support policy makers, NGOs, industry and society.

Keywords: biodegradable polymers, half life assessment, plastic pollution, bioplastics, international standard tests

\*Speaker

# Nanoparticles produced from the degradation of biodegradable microplastic severely affect aquatic microorganisms

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Nowadays, the ecological impact of microplastics and nanoplastics in freshwaters is not fully understood. Since biodegradable plastics undergoes abiotic hydrolysis, here, we have investigated the effects of nanoplastic (< 100 nm) released from microplastic particles of different biodegradable polymers towards three organisms representative of freshwaters, the filamentous cyanobacterium *Anabaena* sp. PCC7120, the green alga *Chlamydomonas reinhardtii* and the macroinvertebrate *Daphnia magna*. These nanoplastics induced a significant decrease in cellular growth in the two photosynthetic organisms and in the mobility of *Daphnia magna*. After abiotic incubation, in liquid suspension, of each biopolymer microbeads, the supernatant resulted to be very toxic to the three microorganisms, but upon ultrafiltration, only a mild toxicity remained. We also performed mechanistic studies of the toxic action by using a set of fluorescence dyes. We performed the physicochemical characterization of nanometric fragments of the biopolymers by DLS, NTA, FTIR and SEM. In conclusion, nanoplastic realised from biopolymer microbeads exhibited toxicity towards aquatic organisms due to their direct interaction with the cells. Thus, our findings indicate that biodegradable plastics may not be as harmless as previously suggested.

*Keywords: Biodegradable microplastic, toxicity, impacts, nanoparticles, freshwater*

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## **November 21st – Panel 10.3: 8h30 - 10h (AGH), Panel chaired by Gunnar Gerdt.**

### **Effects of nylon microplastic on development and energy reserves in coldwater copepods**

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Microplastic debris is a pervasive and widespread pollutant that poses a risk to aquatic biota and healthy marine ecosystems. Copepods are an abundant and ecologically important class of zooplankton, common to marine ecosystems across the globe. Field studies and laboratory exposures have identified that copepods readily consume microplastic particulates. In the copepod *Calanus helgolandicus*, prolonged exposure to polystyrene microbeads resulted in significant reductions in feeding, egg size, hatching success and survival. We hypothesise exposure to microplastics reduces feeding in copepods, resulting in energetic shortfalls for which lipids can act as a proxy. The coldwater copepod *Calanus finmarchicus* is a keystone species, common to the North Atlantic. During maturation, these copepods rapidly build-up their wax-ester store (oil sac); this lipid reserve is essential to the copepod's buoyancy regulation and energetic budget when overwintering, and is of high nutritional value to predators. Following a 48-hour acclimation period, juvenile *C. finmarchicus* were incubated in natural seawater containing a mixed assemblage of cultured algae (control), with the addition of either nylon granules (10-30  $\mu\text{m}$ ) or fibres (10x30  $\mu\text{m}$ ) at a concentration of 100 microplastics  $\text{mL}^{-1}$ . Algal ingestion rates and developmental stage were monitored throughout the exposure period, while prosome length and total lipids (mg) were assessed following the six-day experiment. No significant differences in growth or sex-differentiation were identified. We did however observe that juvenile copepods moulted into adults significantly earlier (ANOVA,  $P < 0.05$ ) when exposed to microplastic, and juvenile copepods exposed to nylon granules showed significantly reduced lipid mass in comparison to controls (ANOVA,  $P < 0.05$ ). We discuss the impact microplastic exposure can have on feeding and energetics of animals, in relation to the individual and marine food web as a whole.

Keywords: Copepod, zooplankton, effects, ecotoxicology

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# Microplastics prevail at all ocean depths of the HAUSGARTEN observatory (Arctic)

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Although recent research indicates that microplastic (MP) has spread to all marine ecosystem compartments from the sea surface to the deep sea, our knowledge about the distribution through the water column is still limited and largely based on model runs. To fill this gap, we deployed WTS-LV large volume pumps at four different depths (sea surface, ~ 300m, ~ 1000m, near seafloor) at five stations of the HAUSGARTEN observatory (west of Svalbard). These pumps filtered 218–560 litres of seawater during each deployment with 10 µm mesh metal filters. Additionally, sediment was sampled at the same stations to understand accumulation mechanisms of MP in the sediment. Our analyses of water column samples using iFTIR spectroscopy resulted in 0–1373 MP m<sup>-3</sup>, comprising 15 different polymer types. MP concentrations in the sediment were found 1,200–33,000 times higher than in the water column. As the concentration measured within sediments are cumulative numbers since 1950's, hourly sedimentation rates of MP were estimated for each station (1 – 57 MP m<sup>-3</sup> dry sediment) to obtain comparable values. The northernmost station, which is located in the transition zone between the open ocean and sea ice, harboured the highest mean concentration (416 MP m<sup>-3</sup>) through the water column, and polyamide accounted for the largest proportion. The surface waters had the highest MP concentrations at all stations with a decrease towards the sediment. Our results will be compared with trends in the vertical distribution of organic particles and discussed in the context of prevailing water masses and sea ice coverage. Still, our preliminary results highlight that noticeable amounts of MP are present throughout the water column, Earth's largest biome, which has been largely neglected in previous estimates of plastic in the world's oceans.

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Keywords: Arctic, HAUSGARTEN, water column, sediment, microplastic, ice, iFTIR, polymer, plastic, pollution, large volume pumps

# An investigation of microplastic contamination in the Arctic Central Basin

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Polar regions are among the most remote environments on planet earth. Despite this, anthropogenic contaminants such as microplastics have entered these ecosystems. In order to assess the potential threats that microplastics pose to marine organisms that inhabit or depend upon polar ecosystems, assessments regarding abundance, distribution and composition of microplastics are important. The present study provided an assessment of microplastics in various environmental compartments of the Arctic Central Basin (ACB). During the Arctic Ocean 2016 expedition onboard icebreaker Oden, microplastics were assessed in (i) sub-surface waters, by using the bow water system of the vessel (single depth of 8.5 m) and the CTD rosette sampler (multiple depths, 8 – 4400 m), (ii) surficial sediments, by sampling cores retrieved using gravity and piston corers (depths, 900 – 4400 m) and, (iii) sea ice cores and water beneath the ice floes. Following sample processing, visual inspection was conducted and potential microplastics were analyzed using Fourier Transform Infrared Spectroscopy. Microplastic abundance in the Polar Mixed Layer (PML) ranged between 0 – 8 particles m<sup>-3</sup> and in the other water layers between 0 – 375 particles m<sup>-3</sup>. Microplastics were present in 7 of the 11 surficial sediments samples from the ACB. Microplastic concentrations in sea ice cores (n = 26) were orders of magnitude higher than was recorded in the water column. Results from this study indicate that microplastics are pervasive in the various environmental compartments of the ACB. These findings suggest that in this oceanic basin (i) microplastics are being vertically transported out of surface waters and, (ii) sediments are a potential sink of microplastics while sea ice is functioning as an intermediate sink. Such findings are particularly relevant as they provide information regarding the concentrations and types of microplastics that polar organisms are exposed to in the ACB's various environmental compartments.

Keywords: Microplastic, Marine debris, Arctic Ocean, Water Column, Sediment, Sea ice

\*Speaker

## Blown to the North? Microplastic in snow fallen out from the atmosphere of Europe and the Arctic

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There is a 99% mismatch between plastic debris estimated to enter the oceans and empirical evidence pointing to yet unaccounted sinks. The FRAM pollution observatory was installed to quantify plastic pollution in different ecosystem compartments to identify hidden sinks and pathways in an area of increasing pollution. Indeed, our first analyses showed enormous quantities of microplastics  $\leq 25$   $\mu\text{m}$  in both Arctic sea ice and sediments from the deep sea posing the question: How is all this plastic transported so far to the North? The importance of atmospheric transport was recently stressed by reports of microplastic in the atmosphere of Paris and Dongguan city. Here, we analysed snow samples from ice floes in the Fram Strait (2016/17) and from snow collected on Spitsbergen, Helgoland, Bremen and the Swiss Alps (2018) to assess the role of this potential pathway of microplastic to the North. Identification of particles was carried out by i-Raman and FTIR imaging. Microplastic particles appeared to be in all samples analysed but polymer composition varied. As with previous data, the sizes of particles were mostly in the smallest size range. The results are discussed in the context of data from other ecosystem compartments.

Keywords: Atmospheric fallout, Arctic, snow, pathway, distribution, microplastic, transport

\*Speaker

## Determination of temporal changes of POP sorption and additive release as well as spectrometric characteristics to a variety of polymers under Arctic marine conditions

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As part of a long-term field experiment within PLASTOX, conducted at marine locations across Europe, a range of different virgin polymer pellets, post-use polymers (LDPE, PP, PS and PET), as

well as marine litter-derived microplastic particles, were deployed underwater for up to 12 months in the small boat harbour of Tromsø, Northern Norway. The deployment device consisted of an empty stainless steel canister, with the various plastic types placed in reusable, empty 'teabags' made of PP, placed separately in nylon netting. Sampling was conducted 1 week, 1 month, 3 months, 6 months and 12 months after deployment. Hydrophobic persistent organic pollutants such as PAHs, PCBs, DDTs, PBDEs and pesticides that had become associated with the plastic were measured and their adsorption kinetics in seawater under Arctic conditions established. Simultaneously we also measured the changes of FTIR- and DART MS signals over time, documenting the change of the IR- and MS spectrum due to weathering. The results will be presented at the conference. For the determination of sorbed POPs, samples were extracted using ultrasound and non-polar solvents, followed by GPC and SPE clean up prior to chemical analysis and quantification by GC/MS/MS and GC/qMS. The release kinetics of common plastic additives, including phthalates, organophosphate esters, bisphenols and perfluorinated chemicals, were estimated from four types of post-industrial virgin pellets (LDPE, PS, PVC, PET) according to the same sampling protocol. Chemical analysis was performed using either GC/MS or LC-QTOF. Results show that HCB and PCBs represented the dominant pollutant classes adsorbing to all of the different polymer types, but at concentrations that are more than 10-times lower than those previously reported. However, equilibrium between pollutants and the polymers was not reached during the deployment period, indicating that Arctic conditions may result in different sorption kinetics than observed in temperate regions. .

Keywords: adsorption, leaching, weathering, Arctic

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## May waterborne polyethylene affect gilthead sea bream eggs hatching and hatchlings survival, by inducing changes in lipid metabolism?

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Polyethylene (PE) was recorded as one of the most frequent polymers amongst microplastic marine debris collected within zooplankton samples in Portuguese coastal waters, its average abundance varying from 0,01 to 0,32±0,3 cm<sup>3</sup>/m<sup>3</sup>. Polymers, such as PE were suggested to induce toxic effects in fish exposed to microplastic debris per se or by leaching chemicals that are

incorporated into plastics during their manufacture or adsorbed in contact with polluted seawater. Fish exposure has been previously tested either through diet or confused and ingested as "prey", but also in fish just exposed to "microplastic-polluted" water, with no clear signs of ingestion. In the present study, we exposed gilthead seabream eggs at the morula stage and newly hatched larvae to 100-200µm PE microbeads, at 0,32 and 0,64 cm<sup>3</sup>/m<sup>3</sup> to study the effect of waterborne PE on hatching success and hatchlings potential to survive, by evaluating: 1) hatching rate and eggs and early stage larvae survival, when exposed to PE "polluted" water; 2) utilization of yolk and oil droplet nutritional reserves during embryonic and early larval development, with an emphasis on lipids and fatty acids metabolism (FA), in particular on polyunsaturated fatty acids (PUFAs) which are known to be essential to fish larvae.

Our results point for a non-significant effect of the tested waterborne PE concentrations on either eggs or hatchling survival, hatching rates, oil droplet absorption or stress resistance in mouth-opening larvae (3 days after hatching). Our preliminary results point for a non-significant effect on lipid metabolism in mouth-opening larvae. However, further analysis on other developmental stages will be carried, since yolk lipids are known to be an important energy source throughout the embryonic and early larval development and its utilization was shown to have a strong impact on the marine larvae further capacity to swim, feed and survive.

*Keywords: fish larvae, hatching, survival, lipid metabolism, polyethylene*

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## **November 21st – Panel 11.1: 10h30 - 12h30 (AGH), Chaired by Juliana-A. Ivar-do-Sul and Jesús Gago.**

### **Microplastic release by Wastewater Treatment Plants and study on ecotoxicological effects induced by virgin microbeads on zebra mussel *Dreissena polymorpha***

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The role of Wastewater Treatment Plants (WWTPs) as entrance routes for microplastics (MPs) to aquatic environment are largely unknown worldwide, as well as their impact on fresh-water organisms. In this study we quantified and characterized, for the first time in Italy, MPs in both sludge and wastewaters in one of the greater Northern Italy WWTP, using the Fourier Transform Infrared Microscope System (iFT-IR). Despite the high MP removal efficiency (83%) of the WWTP, we calculated a release of about 160,000,000 debris/day in the receiving water-body, mainly represented by polyesters, polyamide, polyethylene and polyacrylates. The great amount of MPs that end up to sludge ( $113 \pm 34$  MPs/g dry weight) leads to suggest a possible MP translocation from aquatic to terrestrial environment due to the use of WWTP sludge in agriculture as fertilizer. Considering the high MP amount reaching the aquatic environment, we evaluated for 6 days both the uptake and chronic toxicity of two different mixtures (MIX 1 with  $1 \times 10^6$  MPs/L and MIX 2 with  $4 \times 10^6$  MPs/L) of virgin microbeads on the freshwater filter-feeder zebra mussel *Dreissena polymorpha*. The uptake of these MPs in the tissues of mussels was investigated using confocal microscopy, while chronic toxicity was evaluated every three days, from day 0 (basal level) to day 6, through a battery of 12 biomarkers of cellular stress, oxidative damage, neuro- and genotoxicity. We observed a great number of MPs in the gut lumen, as well as their presence in mussel

hemolymph and accumulation in some tissues. MPs induced a low alteration of the oxidative status in exposed specimens, with a significant modulation of catalase and glutathione peroxidase activities, and a significant increase of dopamine (Figure 1), probably associated to a reduction mechanism of MP uptake across the mussel inhalant siphon.

Keywords: Microplastics, Wastewater Treatment Plants, Freshwaters, Chronic Toxicity

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## Sources of microplastics pollution into the marine environment: importance of wastewater treatment plant and coastal dump

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Wastewater treatment plants (WWTPs) effluent and coastal dumps constitute an important source of microplastics (MPs) input to the aquatic environments. The objective of this study was to assess the amount and types of plastic polymers found along a defined distance gradient from the "EDELWEISS" WWTP outfall in Le Havre harbor and a coastal dump located nearby. Wild mussels, *Mytilus* sp., were collected at five different sites situated near, downstream and upstream the sewage treatment outfall with a respective distance of 50 m, 1.1 km, 1.6 km and 5.3 km and one site near a coastal abandoned dump. Water from all sites including the WWTP effluent was filtered on sieves with different mesh sizes: 500 µm, 200 µm, 80 µm, and 20 µm. Sediments were also collected and analyzed. The results show the occurrence of MPs with various shapes: foams, beads, fragments, and fibers that were classified along their polymer nature using micro-Raman spectroscopy. Their amounts and occurrence in mussels indicate the latter ability to accumulate plastics and fibers depending on the water dispersion and on the distance from the outfall. The highest amounts of MPs were found in both water and mussels found near the coastal dump which, therefore, contributes more than the WWTP effluent as routes of MPs to the marine environment.

Keywords: Microplastics, Wastewater Treatment plant, coastal dump, mussels, microRaman

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## Microplastic dynamics in agricultural systems: Insights from Spanish and Canadian case studies

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Whilst the majority of microplastic research has been focused on marine contamination, greater attention has recently been given towards freshwater systems. Yet, we still know very little about the sources of microplastics to these environments. Wastewater treatment plants are capable of trapping the majority (up to 99%) of microplastics. However, many of the captured particles are concentrated into the solid sludge, which is often applied to agricultural soils as a fertilizer. Hence, soils in agricultural areas are expected to represent significant environmental reservoirs of microplastic. Moreover, these soils may represent a source to freshwater systems that ultimately transfer particles to the marine environment. The Water JPI-funded IMPASSE project addresses several gaps in our understanding of microplastic contamination and release associated with agricultural systems. Sampling has been undertaken in river catchments in Spain (Henares; Guadalajara/Madrid) and Canada (Beaver and Orillia; Ontario). Microplastics were quantified in sewage sludge, agricultural soil, water and fluvial sediments across multiple sites to establish the role of sewage sludge as a source of microplastics in the catchments. Control fields – where sludge has never been applied – were used as an environmental background. Microplastics were extracted following a rigorously-tested isolation procedure and characterised using FT-IR microscopy. The results highlight the dynamics of microplastic particles within agricultural systems and river catchments. This represents a crucial step towards the quantification of microplastic sources and fluxes.

Keywords: microplastic, agriculture, rivers, runoff, freshwater, sewage sludge, wastewater

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## Synthetic fibers affecting soil animals - soil animals affecting synthetic fibers

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Synthetic textile fibers have been identified as one major type of microplastics introduced into the environment by humans. In wastewater treatment plants, most of the fibers released through laundry end up in sewage sludge, which in many countries is used as soil fertilizer in green spaces and agriculture. Despite this vast flow of microplastics in soil, the effects of synthetic fibers in the terrestrial environment are still poorly known. We studied the effects of polyester (PET) fibers on earthworms (*Eisenia andrei*), enchytraeid worms (*Enchytraeus crypticus*), springtails (*Folsomia candida*) and woodlice (*Porcellio scaber*). We also compared the effects and accumulation of two different size classes of PET fibers and investigated whether the size of the egested fibers differs from those introduced into the exposure medium (soil or food).

Our results show that synthetic fibers can affect soil animals, but the effects are slight and sometimes unexpected. Furthermore, it was not only the fibers affecting the animals, but also the animals affecting the fate and size of the fibers. We found that soil animals, particularly earthworms, ingest fibers and in this way can serve as a route of microplastics to the above-ground food web. Our preliminary results also suggest that earthworms can transform the fibers to smaller pieces. These shorter fibers may, in turn, be available for smaller fauna and potentially pose greater risks to the environment. This study is part of the project IMPASSE – Impacts of Microplastics in Agro Systems and Stream Environments

Keywords: soil, fiber, fauna, animal, ecotoxicology, effects, accumulation, earthworm, enchytraeid, springtail, woodlice

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## Microplastics in urban stormwaters – designing a method to evaluate the microplastic discharges via stormwaters

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Urban stormwaters have been suggested to act as a pathway for microplastics to the aquatic environment. At present there is very little actual knowledge of microplastics in stormwaters and the microplastics concentrations on stormwater discharges. In addition, the lack of a standardized method for sampling the microplastics in stormwaters complicates the comparison of the results obtained from few studies. In this study, we developed a flow-based high-volume sampling concept

that gives event mean concentrations for the microplastics from sampled rain events. The sampling concept is based on flow-based pumping and in-situ fractioning. Sampling concept is given in Figure 1.

The samples are collected from the stormwater stream in the stormwater well. First, the V-dam is installed to the discharge side of the well. This makes the surface level of the stormwater stream in the well proportional to the flow rate of the stream. To determine the flow rate in real time (delay < 10 ms) the surface level is followed by using level transmitter. Programmable Logic Controller (PLC) collects and saves this information and uses it to control electrical pump with frequency converter. Pump is used for creating sample flow proportional to the stormwater flow. Also sample flow is saved to the PLC. This information is then stored to Cloud Server and can be accessed with PC either real time or later. PC programme and phone app is developed to follow and control the process.

The sampling will performed repeatedly from one site with well known catchment area. The quality of the stormwater is known to fluctuate due to several factors (e.g. land type and use, intensity and depth of rainfall, antecedent weather conditions), hence it is required to sample adequate amount of rain events to ensure reliable estimation of the total microplastic discharge.

Keywords: microplastic discharge, urban stormwaters, sampling device

\*Speaker

## Industrial compost as an important source of microplastics

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Composting and aerobic treatment are excellent end-of-life options for treating biogenic waste and can significantly improve waste management efficiency as well as reduce environmental burdens (e.g. green house gas emissions). In well developed waste management systems organic waste is routinely collected from households and other sources. The collected material however normally contains a certain level of plastics which enter the process and is a source of contamination. In our study we analyzed four samples of industrial compost for presence of plastic particles. Two methods were used in parallel: the classical "manual" method as routinely used in microplastics analysis, and an automated density-based microplastics sediment separator (MPSS) by the general method

described in Imhof et al. (2012). Infra red spectroscopy was used to determine the composition of particles. Plastic particles were found in all samples at an average estimated concentration of 0,12 wt.%. The majority of particles were composed of polyethylene followed by polypropylene. Fibres were the most numerous particles representing 74 %, followed by fragments. The majority of fibres were composed of PP and PET.

Keywords: plastic pollution, microplastics, compost

\*Speaker

## Release of synthetic microplastic fibres from domestic washing machines: Effects of fabric type and washing conditions.

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Washing clothes made from synthetic materials has been identified as a potentially important source of microscopic fibres to the environment. Our study examined the release of fibres from polyester, polyester-cotton blend and acrylic fabrics. These fabrics were laundered under various conditions of temperature, detergent and conditioner. Fibres from waste effluent were examined and the mass, abundance and fibre size compared between treatments. Average fibre size ranged between 11.9–17.7  $\mu\text{m}$  in diameter, and 5.0–7.8 mm in length. Polyester-cotton fabric consistently shed significantly fewer fibres than either polyester or acrylic. However, fibre release varied according to wash treatment with various complex interactions. We estimate over 700,000 fibres could be released from an average 6kg wash load of acrylic fabric. As fibres have been reported in effluent from sewage treatment plants, our data indicates fibres released by washing of clothing could be an important source of microplastics to aquatic habitats.

Keywords: Microplastic, Microfibers, Plastic pollution, Effluent, Laundry

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## Shipyards: a source of microplastic and micro-paint particles spreading to the marine environment

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Microplastic (MP) pollution affects both marine and freshwater environments, and land-based sources are suspected to contribute with a large amount of discharged plastic waste to the environment. Beside MP, also the presence of micro-paint particles (MPP) have been recently reported. MPP mainly originate from the degradation and abrasion of painted surfaces, such as building and/or ship-paint materials. As many paints also contain organic compounds and heavy metals used as biocides, these particles are potentially even more harmful to the environment than MP. Here we present data obtained from the analysis of soil and sediment samples collected in a recreational boat facility located on the shore of the Limfjord, the biggest transitional environment of Denmark. We performed a multi-step sample preparation, involving air assisted flotation with high density zinc chloride ( $\rho = 1.9 \text{ g cm}^3$ ), enzymatic treatment, Fenton's reaction (catalyzed oxidation), and two phase separation. Then, we analysed the processed samples ( $<500 \mu\text{m}$ ) using FPA- $\mu\text{FTIR}$ -Imaging and processing the data with MPhunter, a dedicated software for automated MP analysis. All the analysed samples were contaminated by MP, and most of them contained also MPP. We measured the highest concentration in proximity to the shipyard area (Site 1 -  $46182 \text{ part. kg}^{-1}$ ) and the relative harbour (Site 2 -  $175636 \text{ part. kg}^{-1}$ ). Besides the most common polymers, we detected a higher concentration of MPP at Site 2 (44% of the total synthetic particles), located inside the shipyard, where the boats are laid up for winter. We successfully extracted and quantified microplastic and micro-paint particles in a recreational harbour area, highlighting that the harbour areas contribute actively to the production, accumulation and spreading of MP and MPP to the surrounding area, ultimately also affecting the marine environment. We are currently investigating the potential correlation between the MPP abundance and the biocides (organic/inorganic) concentration.

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## **November 21st – Panel 11.2: 10h30 - 12h30 (AGH), Panel chaired by Salud Deudero.**

### **Sampling microplastics –a comparative study of a manta trawl and a filtering pump**

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A wide variety of methods have been developed and applied to sample microplastics (MPs). It is important to understand how the methods differ in order to allow for comparisons between different studies, to develop monitoring programs and to choose which method to use for a specific research question. We therefore tested two commonly used methods; a manta trawl and a filtering pump. During one day of sampling at the same spot ten replicates were taken with the trawl (~ 60m<sup>3</sup>) and six with the pump (20 m<sup>3</sup>). The trawl samples had between 9 and 33 MPs, which gave an arithmetic mean of 0.32 MPs/m<sup>3</sup>. The pump had between 0 and 8 MPs per sample rendering a mean of 0.17 MPs/m<sup>3</sup> and it was noted that expanded cellular plastics were sampled more efficiently with the trawl. The results for both methods highlighted the importance of sufficient sample volume, in order to decrease fluctuations in counting statistics. The probability of false null-values increase with a lower true value of numbers of particles and it was seen that this starts to have a significant effect below five particles per sample. Regardless of which method that is used, it is therefore crucial to sample a sufficient number of particles to permit comparisons of spatial, temporal or compositional differences. To further increase comparability in future monitoring studies, we also propose a systematic categorization of plastic particles and other types of microlitter.

Keywords: sampling methods, trawl, pump, sampling volumes

\*Speaker

### **Distribution of microplastics in the mixed layer; results from the Volvo Ocean Race**

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Guteunst \*

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There are large gaps in the knowledge of the fate and distribution of plastics once the plastic has entered the ocean. This is in part due to the vastness of the ocean, the probable uneven distribution of plastics and the challenges in accurately estimating the concentration of plastics. Here we report on a campaign that involved sampling for micro-plastics from two yachts racing around the world in the Volvo Ocean Race. The race started in Alicante in October 2017 and ended in den Hague in June 2018, providing a unique snap-shot of micro-plastic distribution along the race track. The project is a cooperation between the science project, the Volvo Ocean Race and the sponsors of the instrumentation, - the Volvo Cars Group. Samples for microplastics were sampled on 3 different mesh-size SS filters while the boats open the through-hull fittings to run the water maker, allowing for integrated samples over that time- period. The filters were brought to the lab in Kiel and analyzed in a flow-through system with a Raman spectrophotometer.

We found micro plastics in almost all samples along the race track, with the highest concentrations on the south European coast and Mediterranean, as well as in the South China Sea. On several other locations we found higher than average microplastic concentrations that seems to be tied to major ocean currents, suggesting a remote source. Here we will report on experience with the sampling and analytical instrumentation, report on the distribution of the microplastic along the race track and provide some initial ideas on the source of the plastic using numerical modelling. We will also report on the utility of racing yachts as platforms for oceanographic measurements, including micro plastics.

Keywords: microplastic worldwide distribution mixed, layer

\*Speaker

## Microplastics at the first 200 meters of the water column in the Atlantic Ocean

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The presence of microplastic at open ocean is a well documented fact. But they have been determined basically in the first 5 meters of the water column or near the seabed. The buoyant microplastics more abundant are of polyethylene (PE) and polypropylene (PP) composition, with a density significantly lower than seawater density. But there are other kind of plastics, as nylon of fishing nets or fibers which have a density similar to seawater and due to their size, shape and composition, can appear hundreds of meters below seawater surface. Taking into account that

fishing nets represent a high percentage of marine pollution (Lebreton 2018), the possibility to find these small fragments on the water column increase.

Based on this idea, they took place there specific sampling of microplastics at the European Station for Time-Series in the ocean (ESTOC). This station is placed at open ocean at 100 km to the North to the Canary Islands, on the subtropical gyre of the North Atlantic ocean. Sixty liters of seawater were filtered by sample with a net of 100  $\mu\text{m}$  pore size. It was sampling at 0, 50, 100 and 150 meters depth at three different scientific cruises between April 2017 and March 2018. At all sampling done it was observed the microplastic presence at every depths. Microplastics found were basically small fragments of fishing net, lines, paint chips and fibers. This preliminary study supposes that can exist an undetermined small size plastic amount with neutral buoyancy which are not being taken into account at prediction models, underestimating the plastic tons existing at marine environment.

Keywords: deep water, ESTOC, under the surface, POPs

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## Floating microplastics in the Western Mediterranean Sea and their export by marine aggregates

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Pollution by large-sized plastics and microplastic debris is widespread in all Earth environments, also threatening marine ecosystems worldwide. The view of the Mediterranean Sea as one of the most impacted regions in the world by plastic litter has been recently confirmed. However, coastal regions in the Western Mediterranean Sea have been omitted in large-scale surveys in quantifying microplastics and little is known about their dispersal patterns and potential inputs. Furthermore, the discovery of microplastic incorporation into marine aggregates underlines the need to quantify and characterize aggregated microplastics in the marine environment. Whereas the deep-sea has been suggested as a major sink for microplastic debris, the pathways for microplastics to reach such remote environment remain poorly assessed. In this study we determine the abundance, chemical composition, color and size parameters of free floating loose and naturally aggregated microplastics from samples taken in the Western Mediterranean Sea offshore Spain, from the Gulf of Lions to the Alboran Sea. We report microplastic abundances of  $0.100 \pm 0.048$



items m<sup>-2</sup>, with maximum values up to 0.5 items m<sup>-2</sup>, comparable to those found previously in the Mediterranean Sea, which are subject to temporal changes as a function of human activities in the areas of influence and shifting meteorological conditions. Our results show maximum abundances of microplastics around 1 mm in length and with large surface areas in marine aggregates, which represent 41% of all microplastics floating at the sea surface. Marine aggregates would behave both as floats for high density polymers, such as nylon or polyethylene terephthalate, and ballasts for low density microplastics, including polyethylene, polypropylene and polystyrene. Further research and monitoring efforts are needed to understand the mechanisms controlling aggregate formation and sinking, which may explain the removal of microplastics from the sea surface.

Keywords: Microplastics, Marine aggregates, Mediterranean Sea

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## PLASTICS AND EPIPLASTIC COMMUNITIES IN SURFACE WATERS OF THE SOUTH ATLANTIC AND AUSTRAL OCEANS

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Even though marine plastic pollution has been the focus of several studies, when considering the knowledge gaps for many regions of the oceans it is likely that current estimates of their concentrations and impacts are underestimated. Our study aimed to quantify and characterize plastic debris in oceanic surface of two regions, Brazil and Antarctica. Sampling was conducted using manta net (330µm mesh) surface trawls at 10 points along the Brazilian coast (26° to 34° S) and 12 points around the Antarctic Peninsula (61° to 64° S). Plastics were counted and classified in terms of type, size, color and chemical composition. The diversity of epiplastic communities was evaluated through morphological (Scanning Electron Microscopy (SEM) and molecular (DNA metabarcoding) methods. Using Illumina MiSeq High-throughput sequencing a multi-barcode approach was used to characterize the full diversity of life associated with plastics, targeting prokaryotes (16S), eukaryotes (18S and COI), and for the first time in any study in oceanic waters, fungi (ITS). Mean surface debris concentration was estimated at 9059 items.km<sup>-2</sup> for South Brazil,

and 1908 items.km<sup>-2</sup> for the Antarctic Peninsula. Most sampled items were microplastics (< 5 mm), including hard and flexible fragments, pellets, spheres and fishing lines. Plastics were found in nine colors, and were composed of polyethylene, polystyrene and polyamide polymers. Based on SEM, diatoms were the most abundant and diverse group within epiplastic communities. However, our molecular data has revealed a much higher and more variable diversity of life associated with plastics at these regions. This work provides the first description of the Plastisphere at oceanic surface waters off Brazil and the Antarctic Peninsula, and has increased our knowledge of the distribution, concentrations and characteristics of plastics and epiplastic communities in the oceans. This is essential information when considering the potential impacts of plastic pollution on marine wildlife and human.

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Keywords: Marine plastic pollution, Epiplastic communities, Metabarcoding

## Monitoring plastic pollution in the Azores Archipelago: from surface down to the deep seafloor and in biota.

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The Azores archipelago is a remote group of nine volcanic islands located off the North Atlantic Subtropical Gyre. Plastic pollution is ubiquitous in the archipelago but knowledge on its abundance and distribution are poorly known. In this presentation, we provide an overview of recent research efforts dedicated to studying the issue in this remote region of the North Atlantic. Surveys aimed at quantifying plastic debris on the seafloor, along the island's coastlines and at the surface demonstrate a high abundance of debris in the region. Furthermore, results on monitoring plastic ingestion by different components of the food-web confirm a high exposure of marine fauna to these pollutants. Finally, preliminary results on the socio-economic impacts associated with marine debris also reveal significant costs for local populations.

Keywords: plastic, remote islands, atlantic, north atlantic gyre

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# Spatial distribution of microplastics in North Sea surface waters and sediments

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It is well known that microplastics (< 5 mm in size) are omnipresent in the marine environment and will hardly degrade but merely fragment over time. Furthermore, standardized and reliable methods to securely detect microplastics are yet to be set. Determining the abundance and identity of microplastics is a crucial objective in microplastic research, especially with regard to requested monitoring. Nevertheless, information on the abundance and composition of microplastics in the North Sea is still scarce particularly concerning the lower-micrometer range (< 500  $\mu\text{m}$ ). Hence, we analysed samples from two complex environmental matrices at 24 stations in the southern North Sea. Surface water samples were collected with a 100  $\mu\text{m}$  net attached to a neuston catamaran and sublittoral sediments were taken with a Van Veen grab. To isolate microplastics (11–5000  $\mu\text{m}$ ) sediments were first subjected to a density separation performed with the MicroPlastic Sediment Separator (MPSS) and a zinc chloride solution ( $\rho = 1.7 \text{ g cm}^{-3}$ ). Subsequently, a highly promising enzymatic-oxidative purification was applied to all samples using newly developed microplastic reactors. This was followed by a state-of-the-art analysis via Fourier transform infrared spectroscopy (FTIR) imaging. This provides information on polymer quantities, types and sizes as well as spatial distribution of microplastics in North Sea surface waters and sediments. Results show that microplastics are present in all of the analysed North Sea samples exhibiting a variety of polymer types, dominated by rubbers, polyethylene, polypropylene and acrylates/polyurethane. Concentrations ranged from 0.1 to  $2.1 \times 10^2$  particles  $\text{m}^{-3}$  in surface waters and from 2 to  $1.5 \times 10^3$  particles  $\text{kg}^{-1}$  of dry sediment. Concerning size, the vast majority of the detected microplastic particles (95%) was less than 75  $\mu\text{m}$  in length. Finally, this study aims at contributing to a basis for future monitoring measures and stresses the need to include microplastics in the lower-micrometer range into these approaches.

Keywords: microplastic quantification, sediment, plankton, extraction methods, density separation, enzymatic, oxidative purification, FTIR imaging

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# Tracing microplastic pollution in the Atlantic Ocean

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Microplastics are considered the most dominant non-degradable particulate contaminant in the marine environment. Yet, abundance, distribution, and characteristics of microplastics in the open ocean and how they change in time and space still require rigid quantification, especially for smaller microplastics. Interactions of microplastics with marine physical and biogeochemical processes are also largely unknown. These fundamental knowledge gaps hinder our understanding of the exposure of marine ecosystems to microplastics, and thus its potential harms. We traced latitudinal and vertical distribution of microplastics in the top 350 m of the Atlantic Ocean along the passage of the Atlantic Meridional Transect cruise (UK to the Falklands) in 2016. The geographical extent of the samples covered all the Atlantic biogeochemical provinces allowing to constrain the importance of biogeochemical factors such as primary production in redistributing microplastics spatially and through the water column. The role of aggregation with particulate organic matter and subsequent downward flux in transporting microplastics to the abyss was established by quantifying and characterising microplastic flux to 3000 m in time-series sediment trap samples from three locations in the northeast Atlantic (PAP-SO) and in the North and South Atlantic Oligotrophic Gyres (NOG and SOG) during the relevant periods. Our depth-resolved water column and sediment trap data account for microplastics of the lower micro-size limit (25 µm to 1.5 µm) which is unprecedented for marine microplastics sampling programmes and critical for understanding microplastic pollution, its fate and biological and environmental risks.

Keywords: microplastics, abundance, downward flux, vertical distribution, ocean biogeochemistry

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## **November 21st – Panel 11.3: 10h30 – 12h30 (AGH), Panel chaired by Lisa Devriese.**

### **Development of a Py-GC/MS method and its application to identify marine microplastics**

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2,3, Rachid Amara 2, Michel Laurentie 4, Ika Paul-Pont 5, Philippe Soudant 5,  
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## **ADVANCES IN HIGH THROUGHPUT ANALYSIS OF MICROPLASTICS**

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Bruzard 1

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The study of microplastic pollution often involves the analysis of a large number of microplastics. Multidisciplinary analyses on microplastics are also tending to develop, thus greatly increasing the number of manipulations. Therefore, providing an overview of plastic pollution takes time and, despite high throughput analysis, remains a major challenge. The objective of this study is to develop a statistical approach: (1) to determine how many microplastics must be analyzed, based on the total number of microplastics, to give a representative view and (2) to calculate the associated error. This work is illustrated through the example of particle size distribution and chemical nature of microplastic data (Fig. 1) from the Tara Mediterranean campaign (2014). This approach was tested in two cases: first on a regional scale (north-west basin) and then on a local scale (manta by manta). In the case of Tara Expedition data, the results show that only 10% of all microplastics need to be analyzed to give an overview of the samples at the global scale (error < 2.6%), and 17.7% at the local scale (error < 10%). This statistical approach is a major breakthrough for microplastic studies. The interpretation of FT-IR spectra is another limitation of microplastics analysis. The interpretation of these spectra is often long and error-prone. To solve these problems, a function has been developed on the R software with machine learning tools. The preliminary results are encouraging and seem to justify the use of this type of tool in the analysis of FT-IR spectra. Finally, the POSEIDON software will be presented. The objective will be to organize the different functions developed (Sizing of the elutriation column, calculation of extraction speeds, random drawing, identification of FT-IR spectra) in a single data-processing tool.

\*Speaker

Keywords: Microplastic, Tara Mediterranean campaign, Statistical methodology, Machine learning, POSEIDON software

## Freshwater compartments: Screening method for polymer identification and mass contents of microplastic particles using TED-GC-MS

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The pathways of plastics, especially of microplastic (MP), in environmental compartments, particularly in aquatic systems, are not well understood. The critical point is the lack of fast, harmonised methods for sampling, sample preparation and sample analysis. These three analytical steps are dependent on one another and must be optimised. In recent years, we developed a method for representative sampling and fast detection of MP in aqueous systems. The sampling in different freshwater bodies is performed in the field with a fractionated filtration system using mesh sizes of 500, 100 and 50  $\mu\text{m}$ . For water with an intermediate or high content of suspended particular matter a minimum of 1000 L has to be filtered. In the lab, mesh sizes of 10 and 5  $\mu\text{m}$  are used for further filtration. Subsequently, the water filtrates of the different particle size classes are sterilised, dried, weighed and homogenised, if necessary. Conventional methods for MP analysis are infrared and raman spectroscopy, giving information on the shapes and numbers of individually identified MP particles. Our focus is on the determination of mass contents of various polymers potentially contained in environmental samples. For qualitative and quantitative MP detection TED-GC-MS is used, a two-step method based on gas chromatography-mass spectrometry (GC-MS) with previous thermal extraction. This method not only enables us to screen the samples for characteristic marker-molecules, thus identifying single polymers, but furthermore allows the calculation of mass contents of individual polymers. In the present work, different freshwater compartments were exemplarily studied to identify containing polymers and calculate their mass content in MP particles.

Keywords: freshwater compartments, microplastic, mass content, TED, GC, MS, polymers

\*Speaker

## Solvent extraction combined with Py-GCMS: A fast tool for microplastics analysis?

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The environmental research on microplastic (MP) is still an evolving research field. While mass-based and particle-based quantification methods become more and more established, extraction methods from complex samples are still diverse and not standardized. In our study we tested the analytical potential of a sequential solvent extraction for five major polymers (PE/PP/PET/PS/PVC) with pressurized fluid extraction from different complex environmental matrices. The extracts were analyzed by pyrolysis gas chromatography-mass spectrometry (PY-GCMS), a mass-based method for simultaneous polymer specific identification and quantification

recently introduced (Fischer and Scholz-Böttcher, 2017).

The analysis of extracts with PY-GCMS bears several attractive advantages. It enables concentration or dilution of aliquots dependent on sample MP load. Replicated measurements are possible even under different PY-GCMS conditions. This neglects the singularity of PY-GCMS measurements caused by its destructive character.

The extraction of different environmental samples revealed matrix reactions at high temperature and pressure conditions, whose possible interferences with the microplastic quantification have to be checked carefully. These matrix reactions increase with rising organic content of the environmental sample. Furthermore, the promising results revealed different extraction optima for the chosen polymers which lead to a sequential extraction procedure.

We emphasize the high potential and restrictions of pressurized fluid extraction as a fast and cheap microplastic extraction method which can be adapted for targeted polymers even for highly complex and polluted samples.

Fischer, M., Scholz-Böttcher, B.M., 2017. Environ. Sci. Technol. 51, 5052–5060. doi:10.1021/acs.est.6b06362

Keywords: microplastic, PYGCMS, solvent extraction, complex matrix

\*Speaker

## Identification of microplastic particles and fibers using vibrational spectroscopy coupled to multivariate analysis

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Identification of microplastic samples based only on optical microscopy can lead to misidentification and, depending on the size of the particles, to over- or underestimation. The identification of microscopic particles comprises two main steps: firstly, the decision whether a particle is a synthetic polymer or not and secondly, the identification of its chemical composition. The spectroscopic identification of samples conducts to a clear assignment of a sample to a certain polymer origin. Vibrational spectroscopic methods like Fourier Transform InfraRed (FTIR)



spectroscopy or Raman scattering spectroscopy are commonly used, thanks to their complementarities. Some vibrational modes are more active in a technique than in the other depending on the molecule symmetry and on chemical groups. To generate reliable results, the comparison with reference spectra database is absolutely necessary to unambiguously identify polymer type. Given the large number of spectra to analyze and since the spectra libraries usually consist of spectra of pure substances, thus spectra obtained from environmental samples are expected to have low congruity compared to reference spectra. The current solution does not allow easy assignment and fast identification of particles. In order to extend this identification, an alternative solution is based on multivariate analysis. The spectra were analyzed using Independent Component Analysis (ICA). It consists to separate multivariate signal into subcomponents, supposing the mutual statistical independence of the non-Gaussian source signals. This method of identifying microplastic particles using multivariate methods is very powerful, as it takes into account the whole spectrum. This method helps to identify particles type by identifying copolymers and plasticizers, and to distinguish plastic particles and fibers from non-plastics. Those approaches will be presented on both Raman scattering and FTIR techniques, and perspectives on fast microplastic identification will be discussed.

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Keywords: Microplastics, Spectroscopic Identification, Multivariate analysis

## Deep Learning vs Classical Computer Vision Techniques for Microplastics Classification

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Two key elements in the monitoring of the amount of microplastics in the oceans is the standardization of sampling protocols, and the development of automatic tools to reduce the time consuming task of counting and classifying the particles. The use of Artificial Intelligence techniques, more specifically the use of Computer Vision, could speed up the processing the microplastics samples, both from the sea and the beaches.

In this work, a comparison between two approaches for classifying microplastic particles is presented (Fig. 1). Five types of particles commonly found in the Canary Island beaches are considered. Three corresponds to plastics: pellet, lines and fragment; and two to non plastics particles: oil and organic debris. The first approach is the Computer Vision classical pipeline which

is made up of three main stages: image preprocessing, feature extraction and finally the classification stage. The classifier is trained using as input the features extracted in the second stage. On the other hand, Deep Learning is considered as the second approach. In this case, an end-to-end classifier is obtained because the three stages of the classical approach are subsumed into the training stage. Thus, only a set of labeled images is used and the method learns the features to extract and also how to combine them.

For the classical approach a set of features based on color, geometry and texture of the particles is fed to a classifier Random Forest, K Nearest Neighbor and Support Vector Machines has been considered. For the Deep Learning approach, a Convolutional Neural Network has been trained because this architecture has shown good results in other classification tasks. The best result is obtained with the Deep Learning approach with 97.4

Keywords: Deep Learning, Computer Vision, Microplastic Classification

\*Speaker

## Microplastics identification and quantification with Py-GCMS – an improved method for reliable weight related data

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Microplastics are one of the emerging contaminants in the marine environment. Numerous studies have been published on their spatial distribution in the marine environment in the last years. Most of these studies used optical and (vibrational) spectroscopic techniques, such as FT-IR and led to particle abundance related data. However, for modelling mass balances of MP and thus the distribution and fate of MP in the different marine compartments, reliable weight related data is essential. Thermoanalytical techniques coupled to gas-chromatography mass-spectrometry have the potential to create these weight related data. A recent Curie-Point (CP) pyrolysis gas-chromatography mass-spectrometry (Py-GCMS) method (Fischer and Scholz-Böttcher, 2017) demonstrated the identification and quantification of 8 environmentally relevant polymers in environmental samples after sufficient sample clean-up. Weight related data was obtained using external calibration curves and successfully demonstrated with recovery experiments of spiked fish samples. Although the used CP-Py-GCMS is promising regarding the quantification of MP in

environmental samples it has some restrictions regarding sample transfer and capacity.

For that reason, we will introduce the adaptation and advancement of the CP-Py-GCMS method to a micro-furnace (MF) pyrolyzer. The MF-pyrolyzer is stated for high reproducibility and comes with stainless steel targets, which offer the eightfold capacity and more convenient dimensions. The ongoing method improvement is associated with a significant enhancement of linearity as well as lower process standard deviations and potential LODs in the lower ng range. These method improvements in combination with an obligate sample pre-treatment for MP pre-concentration allows the detection and quantification of ppb levels in environmental samples.

The reliability of the data is further enhanced by introduction of injection standards for pyrolysis. The data analysis itself was semi-automated using Automated Mass Spectral Deconvolution and Identification System (AMDIS).

Fischer, M., Scholz-Böttcher, B.M., 2017. Environ. Sci. Technol. 51, 5052–5060 doi:10.1021/acs.est.6b06362

\*Speaker

Keywords: Microplastics, Pyrolysis, GCMS, trace analysis, identification, quantification, simultaneous analysis

## Identification and characterization of biocidal microlitter particles from leisure boat and commercial ship antifouling paint, using correlative microscopy (LM and SEM-EDS)

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Microlitter particles is a diverse and heterogeneous group of matter. It is comprised of plastic polymers but also other minute solid anthropogenic materials. The material composition and its chemical entities, together with size and shape is probably the key to the hazard profile of each group of matter in the larger microlitter domain. Limited information on hazard of specific microlitter classes for given species or ecosystem entities is yet available, but likely certain microlitter classes will be more hazardous than others, and one such category that is designed to be toxic is biocidal particles from antifouling boat and ship paints. Antifouling paint come in limited range of colours, but colour alone is not a sufficient descriptor to link particles to source. In addition, chemical composition needs to be targeted in the identification, otherwise similar looking non biocidal paint or coloured plastic particles can be very similar in visual identification. Here is

demonstrated a novel analytical workflow, based on correlative microscopy, where both imaging in light microscopy and scanning electron microscopy (with elemental analysis with energy dispersive X-ray spectroscopy) is acquired on the same coordinate system to allow correlative image analysis on a single particle basis. Both manual and automatic microscopy workflows is demonstrated. Example results from measurements from sampling in both commercial ship traffic lanes, as well as leisure boat harbours in the North Sea and Baltic Sea will be used to demonstrate the method and illustrate the abundance of biocidal antifouling paint particles in relation to other studies. Samples from large microplastics (> 300µm) to small MP (> 10µm) down to nanoplastics will ne shown.

Keywords: Microscopy, spectroscopy, nanoplastics, antifouling paint, paint flakes, boat, ships, SEM

\*Speaker

## **November 21st – Panel 12.1: 14h30 - 16h (AGH), Panel chaired by Annika Jahnke.**

### **Microplastics in marine sediments of the Mississippi River delta (Gulf of Mexico)**

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Plastic is ubiquitous in the oceans and may persist in the marine environment for hundreds to thousands of years, negatively impacting marine organisms. Thus, a better understanding of the quantities, composition and cycling of plastic in the ocean is urgently needed. The current discrepancy between the measured and the modelled size distribution of floating plastic debris reveals a gap in concentration of microplastics ( Here, we study the potential role of the seabed as a sink for microplastics in the Gulf of Mexico. Our study focuses on a depth transect in the Mississippi River Delta (15 m, 50 m, 150 m, 300 m, 600 m and 2,000 m water depth, respectively). Sediment samples were taken during the NICO (Netherlands Initiative Changing Oceans) expedition leg 7 (12.3.2018 - 4.4.2018) with a multicorer (10 cm diameter). The sediment cores were sliced at 5 cm resolution for the first 10 cm. Subsequently, microplastics (> 10  $\mu\text{m}$ ) were extracted from the sediment samples using a solution of  $\text{ZnCl}_2$  (1.4  $\text{g}/\text{cm}^3$ ) and analyzed by light microscopy and Raman Spectroscopy.

Our results provide insights into the accumulation of microplastics in front of a major river delta. In addition, we discuss how microplastic type and abundance change with increasing distance from the river mouth. We suggest that similar studies should be performed in river deltas worldwide to get a better understanding of the fate of riverine plastic input into the ocean.

Keywords: Microplastic, marine sediments, Raman, North Atlantic

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# Concentrations and morphology of microplastic in the Seine River estuary

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Studies on microplastic (< 5 mm, MPs) pollution are scarce in estuaries. Recently, scientists pointed out freshwater as an important source of microplastics to the ocean (Dris et al., 2015) and estuaries are now investigated as zones of primary interest for the transfer and possible accumulation of MPs between freshwater and ocean. Estuarine hydrodynamics and salinity gradient can greatly affect/modify MP size, and spatial distribution compared to lakes and rivers further inland. The present study aims to quantify the levels of microplastic contamination in surface and sub-surface water of the Seine River estuary.

Two campaigns were conducted during May 2017 and April 2018. Three sites were selected along the Seine River estuary. At each point, two types of samples were taken: surface water (upper 15 cm of water column), and subsurface water (first 50 cm of water column). Samples were collected using 300 µm mesh-size plankton net. Net hauls were conducted for 5 min to 8 min, collecting between 5 and 91 m<sup>3</sup>.

In the lab, organic fraction was removed from the samples using chemical agent (SDS), biozymes and hydrogen peroxide (Löder et al., 2017). Then, microplastics were recovered in the supernatant arising from a density separation using a zinc chloride solution (d=1.7 g.cm<sup>-3</sup>). Finally, supernatant was filtered, and filters observed under stereomicroscope. Number, size and form of microplastics were recorded.

First results show concentration between 1.7 and 7.1 particles.m<sup>-3</sup> (Figure 1). Particles exhibited different forms depending on their origin and/or fragmentation. Most of particles were inferior to 1 mm. A MP distribution difference between surface water and subsurface water is observed.

Characterization by Raman spectroscopy of microplastics collected is in progress. This study is part of the Plastic-Seine 2017-2020 project, funded by the GIP Seine-aval.

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Keywords: Microplastic, Seine River, Estuary, Water column

## Microplastics in sediments from stormwater retention ponds

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Stormwater runoff contains pollutants from land surfaces, and microplastic (MP) is one of the problematic pollutant groups. A large part of the urban stormwater runoff is treated in retention ponds prior to discharge. In this study, we evaluated the prevalence of MPs in sediments from seven stormwater ponds with various catchment types, aiming to understand the emission of MPs from different anthropogenic land uses and the sedimentation in retention ponds.

Sediments were collected from seven stormwater ponds with an Ekman bottom grab sampler. For each pond, three sites were chosen randomly and the sediments were combined. To extract the MPs, sediments were pre-oxidized with 30% H<sub>2</sub>O<sub>2</sub> for 5 days. Density separation using ZnCl<sub>2</sub> (1.9 g/cm<sup>3</sup>) was applied afterwards to reduce the inorganic materials. To further remove the organic matter, the floated particles were oxidized applying a Fenton process.

After extraction, remained particles were concentrated in a 5 mL 50% ethanol solution. A subsample of 200 µL was deposited onto a ZnSe window and dried at 55 °C. The window was scanned by FPA-based Micro-FTIR Spectroscopy (µ-FTIR imaging). Spectrum maps were interpreted using MPhunter, with a library containing more than 100 polymer spectrums.

Keywords: stormwater retention pond, sediment, microplastic

\*Speaker

# Process controls on microplastic recontamination in fluvial sediments due to flooding

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Within river channels the sediment bed plays a vital role in mediating transfers of microplastics since they are buried and stored within the sediments on the river bed. During flood events erosion of the bed surface means microplastics buried within the bed are exchanged with the surface and are potentially re-mobilised. However, we have very little grasp on the magnitudes of floods required to mobilise sediment bed such as to re-mobilise microplastics buried within the sediment or the depths to which beds are disturbed during a flood.

A series of experiments were run within a glass-sided, flow-recirculating flume where a sediment bed was seeded with a 1% concentration of microplastic nurdles (between 3-5mm). The sediment bed was exposed to a period of steady flow for five hours and then beds were exposed to a flood wave with either a 2 hour rising limb and 3 hour falling limb or 2 hour rising limb and 8 hour falling limb. Changes to the bed surface were measured using a laser displacement scanner at a 0.1mm resolution prior to the application of the flood wave, after the rising limb and at the end of the experiment. The bed load and microplastic transport rates were measured every half an hour during the steady flow period and at every step of the flood wave.

Discussion concentrates on linking the changes in bed surface topography to the character of the bedload and microplastic flux in response to differing flood wave characteristics. Data shows that as the bed surface develops during the passage of a flood wave a significant hysteretic response develops in both the bedload and microplastic flux but that the magnitude of response is much greater when considering the microplastics.

Keywords: fluvial microplastics, flooding, remobilisation

\*Speaker



## Microplastic particles in the Ebro Delta, Spain: occurrence, composition and sources

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The ubiquitous presence of plastic debris in the marine environment is an emerging concern as they potentially represent a threat for the ecosystems and for the human health. In particular, microplastics - defined as plastic particles sizing less than 5mm - are of concern especially because they can be transferred along the food web. In the Mediterranean Sea, considered one the largest area for plastic accumulation, only few studies have investigated the role of Mediterranean rivers as corridors for microplastics. In this study, we assess and characterize the microplastic pollution in the Ebro Delta River, a transitional system, from freshwater to marine environment. Three different environmental matrices were evaluated (sandy beaches, river bed sediments and river surface waters) to gain better knowledge on the behaviour and fate of these plastic debris. Methods to separate and quantify microplastics were adapted from previous studies.

Microplastics were found in the sandy beaches on the northern edge of the delta, in the river bed sediments, and in the surface waters of the Ebro river, with mean abundance of  $487 \pm 168$  particles·kg<sup>-1</sup> dry weight (DW),  $2052 \pm 746$  particles·kg<sup>-1</sup> DW and  $3,52 \pm 1,37$  particles·m<sup>-3</sup>, respectively. The different types of microplastics (fibre, fragment, film, microbead, foam and aggregation of fibres) were dominated by fibres sizing less than 1mm. This study represents, to our knowledge, the first evaluation of microplastic pollution in the Ebro river basin. The profile of microplastic concentration across the Ebro Delta showed that i) river bed sediments present higher concentrations than sandy beaches suggesting that Ebro's riverbed might be a potential sink for this anthropogenic pollutant and ii) populated areas and riverine influence are factors which increase the presence of microplastics.

Keywords: Microplastics, Mediterranean coastline, transitional environment, Ebro Delta

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# Assessing microplastic abundance and distribution in the surface waters of the Mondego river estuary (Portugal)

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Rivers are recognized as an important source of microplastic pollution in the open sea, however, the fate and patterns of distribution of microplastics in freshwater and transitional systems are still unclear. The present study aims to contribute to baseline information regarding the spatial and temporal occurrence of microplastics in surface waters of the River Mondego estuary (Portugal). During four sampling events in 2017, surface water samples were collected in 5 sites along the estuary and microplastics were extracted after digestion of the organic matter by using a KOH (10%) solution. Microplastics were found in all samples, with  $1.53 \pm 1.04$  n.m-3 on average. A total of 1127 particles were characterized by size, shape, colour and polymer type. The current data showed the absence of a spatio-temporal pattern of distribution of MPs in the Mondego estuary. Potential microplastics were isolated and analysed using Fourier Transform Infrared Spectroscopy (FT-IR) for polymer determination. More than 80 % of the particles were microfibers and the fragments were mainly made of polyethylene and polypropylene, while the anthropogenic fibers were mainly made of polyester but also from natural and semi-synthetic origin (rayon). Microplastics concentration was diverse along the estuary, reflecting various potential sources and sinks such as wastewater treatment plants, tributaries and weirs. This study can provide valuable reference for better understanding microplastic pollution in inland waters and baseline data for the assessment of ecological status of transitional ecosystems.

Keywords: Contamination, Microplastics, Estuaries, Microfibers, FTIR, water.

\*Speaker

## **November 21st – Panel 12.2: 14h30 - 16h (AGH), Panel chaired by TBD**

### **MICROFIBER INGESTION IN OTARIIDS ALONG CHILEAN AND PERUVIAN COAST: A POSSIBLE SENTINEL OF BIOMAGNIFICATION?**

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Recent studies report the ingestion, biomagnification and negative impacts of microplastics (< 5mm) in several species at low and medium trophic levels, suggesting that small plastics, such as microfibers (< 1mm), have the potential to be incorporated in food webs. However, in most marine top predators, such as marine mammals, the presence, and impacts of microplastics has been scarcely evaluated. This work evaluates the presence of microplastics in feces of 3 otariids species (*Otaria byronia*, *Arctocephalus australis*, *A. phillippii*) to evaluate differences in pollution levels depending on the diet, zone and/or species. Fecal samples were obtained from four different colonies located from 15°S to 43°S (Punta San Juan in Perú, Juan Fernández, Chiloé, and Guafo Island in Chile). Microplastics, in particular microfibres, were observed in feces from all the colonies with a lower concentration in Perú (Mean=9 microfibers units per sample) compared to those in Chile (Mean= 26-32) ( $p < 0.05$ ), and lower in sites located closer to the continental coast (Punta San Juan and Chiloé) than further sites (Juan Fernandez and Guafo Island) ( $p < 0.05$ ). The location was the most significant variables for predictive the concentration of microplastics in feces, suggesting that differences in feeding behavior, related to distribution area, might explain the findings in this study ( $p < 0,05$ ; ANCOVA). Juan Fernández (78°W) colony had high values of microfibers, followed by Guafo (74°W), Chiloé (73°W) and Perú (75°W), observed a trend of

increasing microplastic pollution from North-East to South- West, considering that are reports that said that the microfibers concentration in coastal and oceanic areas are highly influenced by currents. These differences in concentrations make otariids potential sentinels for plastic pollution in their food web and environment. Additionally, the non-invasive collection of feces from remote otariids rookeries makes this a simple and effective technique to evaluate plastic pollution in top predator diets.

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Keywords: microfibers, fur seal, sea lion, bioaccumulation

## A STOCHASTIC MATRIX POPULATION MODEL REVEAL SUBTLE, BUT IMPORTANT, POPULATION-LEVEL EFFECTS, OF THE ENTANGLEMENT WITH MACROPLASTICS IN A SOUTHERN FUR SEAL POPULATION FROM NORTH PATAGONIA, CHILE.

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Plastic accumulation on oceans has serious negative effects on marine environments. In this context, the entanglements with macroplastics is one of the main problems for marine vertebrates worldwide. The pinnipeds, a group of marine mammals which is widely distributed across the planet, is especially susceptible to suffer this type of entanglements. In spite of this, entanglement rates in this group is commonly low (< 9%) and there is not clear if the negative effects observed at the individual level (e.g. death, decrease of the frequency and duration of feeding trips, etc.) could scale up to the population level. In this work, we present the results of a 5-years monitoring of a breeding colony of Southern fur seal (*Arctocephalus australis*) from the Guafo Island, Northern Patagonia, Chile. During 2013-2017, we recorded information about key population parameters (e.g. abundance, return rates, fecundity) as well as about entanglements rates. Using this empirical information, we parametrized a stochastic matrix population model structured by age classes (pup, juveniles, adult males, and adult females) in order to predict the population growth rates under five

scenarios: A) without entanglements, B) entanglements rates observed in our study population (i.e. 0.03-2.9%); and different entanglements rates reported in literature for other pinnipeds species, namely: 3) 2.5%, 4) 3%, y 5) 8%. The asymptotic population growth rate (a.k.a. finite rate of increase) was significantly lower (i.e.  $p < 0.05$ ) in the all the scenarios that considered entanglements in comparison with the model that with zero entanglements. Our results show that commonly used population models can be a simple and effective tool to evaluate subtle, but important, negative effects of the entanglement with plastics at the population level.

\*Speaker

Keywords: Pinnipeds, Entanglement, Population Dynamics

## Marine plastic pollution in the Southern Ocean

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Plastic litter and other objects which have been lost or discarded at sea are recognised as a major source of marine pollution. Understanding the magnitude and significance of the effects of marine debris on the Southern Ocean ecosystem requires systematic recording and reporting of relevant data in order to establish baselines and to understand trends. Here we present data on three decades of marine debris monitoring in the Scotia Sea at various sites on South Georgia and at the South Orkney Islands. These data are submitted annually to the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) as part of their marine monitoring programme. We examine (a) trends in the presence of beached marine debris; (b) rates of entanglement in debris of marine mammals, particularly the Antarctic fur seal *Arctocephalus gazella*; and (c) the presence of marine debris in association with flying seabirds, particularly the wandering albatross *Diomedea exulans*.

Keywords: macroplastic, entanglement, long, term monitoring, Antarctic, Southern Ocean, Scotia Sea, beached debris

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## Microplastic Pollution in the Southern Ocean

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It is estimated that there are up to 5 trillion pieces of plastic in the oceans (Erikssen et al., 2014). Microplastic pollution (particles < 5mm) is recognised as a major problem in the world ocean, (Cozar et al., 2014). Antarctica is generally thought of as a pristine and isolated wilderness, free of most of the anthropogenic stressors found in populated regions of the world. However, recent studies in the Southern Ocean have reported microplastics in deep-sea sediments (Van Cauwenberg et al., 2013) and surface waters (Cincinelli et al., 2017). We present our predictions of microplastic contamination in the Southern Ocean, based on quantifiable data from research stations, cruise ships and fishing effort. Our findings suggest that at an Ocean basin level concentrations should be insignificant, but that at point sources the contamination may be high. We report the detection of the first microplastics in shallow benthic sediments close to a number of research stations on King George Island. Worryingly, our predictions of primary microplastic concentrations from local sources are five orders of magnitude lower than levels reported in published sampling surveys, some of which were comparable to levels found in highly populated areas of the world (Erikssen et al., 2014; Adventurescience, 2016). We speculate that plastic contamination originating outside the Southern Ocean is crossing the Polar Front and contributing to the problem.

Keywords: Antarctic, tourism, fishing, research, policy, pollution, marine

\*Speaker

## Beyond 62°S: Plastic litter on the coasts of King George Island, Antarctica

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Plastic is an important and ubiquitous material in our economy and daily lives. However, plastic pollution is also disrupting the functioning of coastal and marine systems worldwide, including

Antarctica. Since the 90s CCAMLR has recognized this threat and promoted research and monitoring. In 2018 SCAR approved an Action Group (PLASTIC-AG) to redouble research on the plastic issues in Polar Regions. Supported by the Uruguayan Antarctic Institute (IAU) since 2016, our project aims to generate an integrated diagnosis of plastic debris in the marine- coastal areas of the Fildes Peninsula (King George Island). To this end, we have studied beached, surface waters, and glacier contributions of macro, mega and microplastic (< 5mm), as well as seabirds plastic consumption. Chemical compositions and POPs concentrations of beached debris were analyzed. This paper focus on beached debris obtained in 900 sampling points (5 campaigns: 2016-2017-2018) in 6 areas of the Peninsula. Each sampling point was geopositioned and defined by the presence of at least one plastic debris on a 5m radius surface. The collected debris were classified, measured and weighed according to the CCAMLR sampling guide for beached marine litter. Debris generated by the functioning of scientific bases (land sources), and particularly thermic isolation requirements, were the most abundant. However, plastic debris from marine activities were also found, as well as some of unknown origin (pieces of rubber, incinerated blocks). These results, and the ones obtained in the other components of our project, aims to generate a baseline dataset to identify plastic pollution hot spots, possible origins, and links with human pressures and activities. This information will contribute to establish monitoring programs in the Fildes Peninsula and thus to CCAMLR initiatives on plastic pollution.

Keywords: Marine, coastal areas, Antarctica, Fildes Peninsula, baseline

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## Plastic pollution around Antarctica – preliminary results from the Antarctic Circumnavigation Expedition (ACE)

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The Antarctic Circumnavigation Expedition (ACE) sampled micro, meso and macroplastic litter around Antarctica from December 2016 to March 2017, with the aim of providing the first comprehensive synoptic survey of the levels of plastic pollution in Antarctic waters. Preliminary results show that the Southern Ocean can be regarded as the ocean least polluted by plastics globally. Only small numbers of micro-fibres were found in 173 beach sediment samples collected

from 12 Antarctic and sub-Antarctic islands visited during the expedition and only 7 plastic particles (identified by FTIR analysis) were retrieved from 33 plankton samples collected around the continent using a 200 µm neuston net. In addition, only 22 macrolitter items (> 2 cm) were visually observed floating south of the Subtropical Front in almost 15,000 km of transect counts. Nevertheless, anthropogenic litter was found in two seabed Agassiz trawls and macroplastic items were recovered from most beach landings, though quantity varied with location. Synthetic microfibres were detected in virtually all bulk and underway water samples collected around Antarctica. Surprisingly, there was no marked gradient in these fibres as we approached continental source areas. Confirmation of the chemical identity of these fibres is still pending, but if they prove to be plastic, they suggest that all the world's surface waters apparently carry low concentrations of microfibre pollutants, at a density of ~ 0.1-1 fibres per litre.

Keywords: Antarctica, Southern Ocean, Plastic Pollution

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## **November 21st – Panel 12.3: 14h30 - 16h (AGH), Panel chaired by Paula Sobral.**

### **Microplastic pollution in upstream river catchments**

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### **An opportunistic monitoring approach for the evaluation of surface plastic debris pollution in Western Mediterranean Sea, Iberian Coasts and Macaronesia**

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Studies have been performed to monitor the abundance of marine plastic debris, mainly floating microplastics. Nevertheless, the existing data sets are fragmented, based on heterogeneous methodologies and didn't allow for now to obtain an exhaustive representation of the geographical and temporal microplastic distribution. We assume that this lack of data is related to the costs to perform the sampling and the time required for sample analysis, mainly smaller particles. This study proposes a simplified way to obtain a reliable data set at a moderate cost, and a methodological approach to extrapolate missing values. Adapting and applying this approach to other microplastic datasets may be able to find a common component to achieve comparability.

First, we built a sample set with the help of volunteer sailboats. Secondly, we focused on

particles whose size exceeds 1mm to reduce massively the analysis time. Finally, we developed a statistical model based on measurements allowing to extrapolate the mass and number of particles whose size is between 0.33 and 1mm. A comparison between the measurements and the model show limited error (fig1B). This approach allowed us to obtain a fine mapping of floating microplastics in the western Mediterranean Sea (133 samples), showing for the first time a good correlation - in terms of geographical distribution - with prediction models presented in different studies (fig1A). Our results in Macaronesia and Iberian coasts (84 samples) provide new inputs concerning plastic accumulation in the North Atlantic subtropical gyre, more specifically as regards its eastern de- limitation. Moreover, a typological analysis (fig1C+1D) of the microplastics reveals a majority of fragments in the two regions but also raises differences: the films are more abundant in the Mediterranean Sea whereas we find more lines in North Atlantic. These typological differences could explain differences in the extrapolation models associated to these 2 regions.

Keywords: Microplastics distribution, Mediterranean Sea, Macaronesia, simplified methodology

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## Citizen Observatories to reduce and identify marine plastic pollution loads

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Plastics in rivers, lakes and inland waters represents an emerging threat for aquatic ecosystems and a major source of marine microplastics, with nearly 80% of marine debris having land based origins. However, the mechanisms and amount of plastics released into freshwater and terrestrial environments have not been clearly identified, and solutions to reduce this load have yet to be developed. Furthermore, more information about the variability of transport mechanisms (rivers, sewage, flooding), relative abundance of type and primary sources of this anthropogenic litter are not well understood. This information is fundamental to determine its impact and to identify successful mitigation strategies to reduce the load and impact on the marine environment. While the scientific community is dedicating much effort on the impacts of plastic on marine ecosystems, the problem of plastic pollution needs to be tackled on land. Through citizen observatories, we show that participatory citizen science can promote public stewardship of local aquatic resources as well as provide robust information on the type and quantity of plastic entering rivers and lakes. Such information can be used to reduce the amount of plastic reaching our seas.

Efforts to solve the plastic problem require a partnered approach between researchers, municipalities, educational institutions and communities. Focusing on the connectivity of all aquatic ecosystems in the emerging challenge of plastic debris in the hydrosphere, we present the efforts of the community of citizen scientists monitoring local water bodies for anthropogenic litter presence and composition. Through a collaboration between municipalities in Tuscany, local communities, University of Siena and international citizen-science initiative FreshWater Watch of the Earthwatch Institute (UK), citizen scientists have been monitoring the presence of macroplastics and ecosystem conditions in the Arno River catchment (Italy) since 2016. These measurements provide new insights into the drivers of plastic loads entering into the Mediterranean Sea.

Keywords: Social change, from mountains to the sea

\*Speaker

## Aerial detection of plastic pollution in the marine environment using a novel, hyperspectral infrared camera on a UAV

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As the inventors of plastic and the designers of its innumerable applications, our collective society is impeccably positioned to minimize the consequences of our now widespread and persistent pollution of the world's oceans. Our research team is developing and deploying a novel, airborne camera to detect plastic items of varying size in the marine environment and to generate solutions to this global issue, while addressing several remaining challenges consistently highlighted by the plastic pollution research community. An objective tool for detecting and mapping debris reports inherently standardized units of environmental contamination, eliminates effort bias associated with observation sampling, and removes the need for time-consuming and nuanced preparation, transport, and extraction of physical samples. The hyperspectral imaging technology is based on Fourier Transform Infrared (FTIR) spectroscopy: a tool commonly used in the laboratory to identify plastic polymers based on their absorption of radiation of various wavelengths. By eliminating the moving parts of this technology, we have designed a modified optical configuration

for a compact payload on an Unmanned Aerial Vehicle (UAV). This deployment strategy increases the feasibility of accessing remote locations while reducing the cost of survey operations. Initial deployments of this novel imaging system will take place in Scotland and in the Arctic region of Svalbard during the coming field season, and will test the equipment in increasingly extreme environments.

Keywords: remote sensing, Arctic, macro, technology, FTIR, optics, imaging, UAV, airborne

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## Experiences and potentiality of a continuous microplastic sampler: performance onboard opportunity vessels to increase oceanic sampling efforts.

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Broad scale monitoring efforts and microplastic sampling in open ocean waters are required to understand distribution, abundance and fate of these particles in the environment. For surface seawaters, towing a neuston net is the most common sampling method; however, the deployment of this infrastructure is not always possible when at sea. The underway water system of Research Vessels (RVs) has been tried for this purpose. However, method validation and recovery checks have not been thoroughly applied, and standardization is essential to ensure the intercomparability of the data reported in the different sampling efforts worldwide. This preliminary study pursues to demonstrate the potential of a continuous microplastic sampler as an effective method to sample microplastics in subsurface oceanic waters, allowing constant sampling without interfering the regular activity of the vessel in which it is set up. This is an important step that increases the chances for ocean monitoring on microplastic pollution with the use of a wider array of opportunity platforms, such as recreational vessels.

To fulfil the main aim of the study, the continuous microplastic sampler was tested and improved along different research campaigns onboard research and recreational vessels in the NE Atlantic. Performed method validation proved the effectiveness for microplastic research com-

paring the samples taken through the intake system of the RVs and samples filtered from the Rosetta. Likewise, the abundance and distribution of microplastics found were also consistent with available literature data. Further trials are required to test the effectiveness and significant difference among the abundance and type of microplastic particles collected using different methods. Ongoing research is being carried out in collaboration with the Sailing Living Lab Project to test additional environmental and sampling factors, and improve the design of the continuous microplastic sampler for its optimal performance.

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Keywords: marine plastic pollution, seawater sampling, continuous monitoring, small, microplastics, method validation, opportunity vessels.

## Microplastics in European Fleur de Sel and Sea Salts – a qualitative and mass-quantitative geographic comparison

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By now microplastics (MP) are almost ubiquitous. Since particle related data are highly relevant for ecotoxicological studies, their high variability in shapes and sizes make data comparison difficult and often speculative. Thermo analytical methods combined with GCMS provide polymer specific analysis of preconcentrated complex environmental samples as well as qualitative and mass-quantitative data, independent of shape or size of MP. This complementary approach enables a more general study of distribution and fate of MPs including modelling. Further improved Py-GCMS [1] allows MP analysis in environmental samples on a ppb level and below. Here results for marine salts from different regions are presented. Marine salts are popular. Especially Fleur de Sel (FdS) is regarded as a pearl in haute cuisine. As almost natural products obtained from sea water marine salts should reflect their local environment and consequently its MP load to a representative extent. Easy available they are an ideal tool for MP studies on a supra-regional scale.

12 FdS and 5 sea salts from European Atlantic and Mediterranean coasts were investigated for 9 environmental relevant polymers. PP, PE and PET were the most dominant MP types in the samples, followed by PS and PVC and infrequent by PC, PUR, PMMA and PA-6. While the mean of sea salts was 37 ig MP/kg ( $\pm$  18 ig) that of FdS was 10fold as high with 386 ig MP/kg ( $\pm$  242 ig) excluding an outlier of almost 2000 ig/kg. This discrepancy is directly related to the unique production process of FdS. The different regions will be compared regarding their plastic composition and quantities.

Besides the potential to reflect the local plastic load the analysis of marine salts underlines the general circulation of marine litter and its prompt return to human menu.

Keywords: Microplastics, Py, GCMS, marine salts, mass, quantitative data, supra, regional co- parisson

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## **November 21st – Panel 13.1: 16h30 - 18h30 (AGH), Panel chaired by Gunnar Gerds.**

### **Analytical approach for the identification and quantification of microplastic particles in aquatic samples by a combination of particle analysis with FTIR and Raman microscopy**

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The quantitative detection of microplastic particles (MP) in an environmental sample in the size range of 1  $\mu\text{m}$  to 5 mm in a reasonable time represents a challenging mission. The presentation will describe the procedures to identify and quantify MP in aquatic samples including the work in MP-free rooms, the use of MP-free equipment and clothes, and the use of blank tests in all steps in the lab. The first part is a three-step vacuum filtration to divide the particles in four fractions between 1  $\mu\text{m}$  and 5 mm. After filtration, the particles are on silicon filters which are suitable for FTIR and Raman measurements. An optical particle search program determines all particles in shape and dimension and stores their coordinates for the subsequent measurements with FTIR and Raman microscopy. Both methods identify the MP on the filter by their chemical structure using spectral databases. Every particle is assigned via the spectrum to a substance. This can be a polymer, in case of a mixed spectrum a polymer with paint/filler, or no polymer. The fractions above 50  $\mu\text{m}$  are measured mainly by FTIR and the fraction below 50  $\mu\text{m}$  mainly by Raman. However, several samples are measured with both methods, because a combination of both spectroscopic methods delivers a complete analysis of MP. The advantage of FTIR is a faster measurement and often a better identification of the polymer matrix in coloured samples; the disadvantage is the detection limit of 20  $\mu\text{m}$  size. The advantage of Raman is the better detection of additives and paints and the identification of particles up to 1  $\mu\text{m}$ ; the disadvantage is the longer measurement time and the appearance of fluorescence in case of not sufficiently cleaned samples. All parameters which influence the measurement and a comparison between FTIR and Raman will be discussed.

Keywords: microplastics, particle analysis, FTIR, Raman

\*Speaker

# Optimising the Workflow for Microplastic Analysis by FTIR Microscopy

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Analysis of environmental samples containing microplastics is essential to determine their prevalence and their impact. A range of analytical techniques have been applied to the analysis of microplastics. Of the techniques adopted, infrared (IR) spectroscopy, and more specifically IR microscopy, has established itself as a primary analytical technique for the detection and identification of microplastics. The microplastics analysis workflow for IR microscopy consists of several steps involved in getting from the raw sample to answers, including the initial sampling through to data analysis. The steps involved may be different depending on the type of initial sample and the amount of sample cleanup required to prepare the sample for infrared (IR) analysis. This paper describes the different types of environmental samples, the sample collection methods, the range of different sample cleanup methods, and then deals more specifically with the best ways to optimise sample filtration for measurement by IR microscopy. The principles of IR microscopy and the different sample measurement modes will be described, comparing and contrasting each type. IR microscopy and imaging experiments can generate significant quantities of data that need to be analysed to get the required information. The different methods for extracting data and information will be explained and suggestions made for best practice.

Keywords: FTIR, microscopy, analysis

\*Speaker

## Measuring the sub 10 $\mu\text{m}$ fraction - Microplastic analysis of particles < 10 $\mu\text{m}$ using $\mu\text{FTIR}$ imaging

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Microplastic pollution has long been – and remains – cause for concern in society. With more research pointing out knowledge gaps on microplastic and its behaviour, the lack of standardisation, reproducibility and comparability of research is becoming increasingly apparent. Data



comparability is not only hampered by sample preparation, but also by difficulties in identifying whether a particle is plastic. Scientific consensus has formed that with decreasing particle size, manual microscope analysis is no longer viable in retaining objectivity and accuracy. Instead, the standard analytical method is moving towards spectroscopic methods such as micro-Fourier Transform Infrared ( $\mu$ FTIR) imaging, which are more reliable and if used correctly, maintain full objectivity. However, in many reviews of analytical techniques,  $\mu$ FTIR imaging has been deemed unable to quantify microplastic particles below 10 - 20  $\mu\text{m}$  in size. In this work, we present results showing that this is a misconception. We demonstrate that data acquisition using modern day equipment can identify particles down to at least 1.3  $\mu\text{m}$ . We show acquisition at different magnifications (7.3x, 12.1x, 36.4x & 60.6x), of the exact same sample which consists of a standard polymer mix containing 8 polymer types. We determine spectral quality of all particles within the area measured based on a set of quality metrics, such as spectral noise and regression coefficient of fit to their respective reference spectra. We moreover demonstrate that acquisition of data can be considerably selective in terms of particle size determined, as this is very dependent on acquisition type and ability of machinery. With higher magnification modes than the standard analytical practice, we find up to 4.6x more particles, of which up to 63% are < 10  $\mu\text{m}$  and 20% under 5  $\mu\text{m}$ . We contend that this poses a significant advancement in analytical capabilities for measuring of biologically relevant size fraction of microplastics.

Keywords: Analytical methods, FTIR

\*Speaker

## nano-FTIR - from defined to environmental (nano)plastic samples

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Recent studies on microplastics in various environments indicate a strong trend to high abundances at small particle sizes of a few micrometres down to the nanometre range. The identification of nanoplastic particles (smaller than 1  $\mu\text{m}$ ) in marine environments and their ecotoxicological effects are matters of high importance and intensifying research. Such particles can pass cell membranes and remain in marine organisms with accumulation effects in the overall food web. So far, very little is known about the formation and distribution of nanoplastics in the environment. One of the grandest challenges is their identification, as methods commonly applied

for single particle microplastics analysis lack the necessary resolution. Nano-FTIR is a novel technique, which combines the nanoscale local resolution of atomic force microscopy with near-field infrared spectroscopy resulting in unprecedented material identification on a nanometre scale. We demonstrate its application in the analysis of defined (nano)plastic samples and those from a marine environment, Arctic sea ice. As implied by Figure 1, there are significant differences between particles of the two types and their environment. An emphasis will thus be on the particularities of the environmental nanofraction in sample preparation and analysis.

Keywords: nanoplastics, nanoFTIR, environmental samples, infrared spectroscopy, scattering type scanning nearfield optical microscopy

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## Comparison of $\mu$ -ATR-FTIR spectroscopy and py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments

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In recent years, many studies on the analysis of microplastics in environmental samples have been published. These studies are hardly comparable due to different sampling, sample preparation, as well as identification and quantification techniques. Here, microplastics identification is one of the crucial pitfalls. Reliable, chemical structure-based identification methods are needed. In this context, vibrational spectroscopic techniques but also thermoanalytical methods are widely established. The presentation will provide a critical comparison of these fundamentally different approaches with regard to analyzing microplastics in environmental samples.

In a blind study, we investigated 27 microplastics particles and fibers of unknown material isolated from river sediments. Successively micro-attenuated total reflection Fourier transform infrared spectroscopy ( $\mu$ -ATR-FTIR) and pyrolysis gas chromatography-mass spectrometry (py-GCMS) in combination with thermochemolysis were applied.

Both methods differentiated between plastics vs. non-plastics in the same way in 26 cases, with

19 particles and fibers (22 after re-evaluation) identified as the same polymer type.

To illustrate the different approaches and emphasize the complementarity of their information content, we exemplarily provide a detailed comparison of four particles and three fibers and a critical discussion of advantages and disadvantages of both methods.

Käppler et al., 2018, Analytical and Bioanalytical Chemistry, <https://doi.org/10.1007/s00216-018-1185-5>

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Keywords: comparison, validation, py GCMS, ATR, FTIR, microplastics analysis

## The use of spiked samples for the validation of microplastic recovery and analysis methods: density separation, enzymatic purification and FTIR analysis

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The quantification and qualification of microplastics in environmental samples requires several steps in order to separate them from samples. The processing can be impacted by several factors: i) sample contamination with plastics stemming from the surrounding environment ii) alteration of microplastics due to the use of chemicals iii) loss of microplastics during density separation or sample transfer. Since the microplastic research is evolving rapidly, there is a general need of more precise methods to quantify them in the environment. Within the framework of the JPIO-Project "BASEMAN", the aim of this study is twofold. In the first place, a reference kit of plastic particles was developed to serve for method validation. The second aim was to use the developed kit to validate a complete workflow, identify the related uncertainties and provide an efficient and accurate protocol for microplastic recovery and analysis. For the first goal, five polymers (PA, PE, PET, PS and PVC) were selected. Irregular fragments with various shapes were obtained by grinding. This reference kit contains some of the most abundant polymers in the environment while covering a large spectrum of densities. Moreover, it consists of three size classes that present different analytical challenges (1 mm, 100 µm and 20 µm). For the second purpose, sediment, plankton and biota samples were spiked using this reference kit. A density separation with the MPSS (Munich

Plastic Sediment Separator) using ZnCl<sub>2</sub> solution is used to remove the mineral particles from the sediment samples. An enzymatic purification using SDS, enzymes and H<sub>2</sub>O<sub>2</sub> is carried out to remove organic material for all types of samples. Particles are then analysed with Focal Plane Array-Micro-Fourier Transform Infrared (FPA-FTIR) Spectroscopy. The recovery rates of this workflow were identified thanks to this experiment. Moreover, it allowed to verify the possibility to detect small particles of 20 µm.

Keywords: Microplastics, Protocol validation, Enzymatic digestion, FTIR, Density Separation, Stan-

\*Speaker

## A freeware tool for the manufacturer independent analysis of microplastics allowing the automated analysis of FTIR imaging data

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The analysis of microplastics is a challenging field and consists of different steps starting with sampling, work up/purification and finally analysis. Each step has its own challenges and for the analysis different spectroscopic methods are available with different data quality and comparability. The state of the art analysis via FTIR imaging allows the analysis of complete filter areas independent of human bias in a short amount of time. For the development of standardized operational protocols (SOPs), comparable data determination is hampered by the different manufacturer software and commercial software available. While an automated analysis approach is available for the Bruker OPUS c Software, it cannot be easily transferred to other systems. To overcome this challenge and allow the harmonization of data analysis we developed the tool MPhunter (see Figure 1a). It allows the analysis of datasets measured on different machines from the manufacturers Bruker, Agilent and Thermo Fisher (further in development). Every spectrum can either be selected by an individual number or a false color heat map. Large dataset with more than 3.6 million can be handled by it with relative ease. The automated analysis approach is included and therefore manufacturer independent. In addition to this ability, it significantly reduces the calculation time from more than 24 hours down to 2 hours for a reference data set (see Figure 1b) containing 1 million spectra. The generated data was benchmark and validated in accordance to the original approach to allow a comparison of results. This new tool will be available as Freeware and allows the standardization of MP data analysis for spectroscopic data for future research.

Keywords: FTIR, automated analysis. \*Speaker †Corresponding author: sebastian.primpke@awi.de

## **November 21st – Panel 13.2: 16h30 – 18h30 (AGH), Panel chaired by Lars Gutow.**

### **First data on plastic litter ingested by seabirds in the Bay of Biscay**

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Trends in the amount and composition of litter ingested by marine animals constitute one of the criteria associated to the Descriptor 10 (Marine litter) of the Marine Strategy Framework Directive (MSFD). Seabirds have been widely used to assess this human impact. In the European Atlantic Area, the Northern Fulmar (*Fulmarus glacialis*) has been the main species used to monitor the ingestion of plastic litter. However, this species is very scarce in the Bay of Biscay (BoB) and potential alternative suitable biomonitor species should be evaluated. Hence, the aim of this study was to assess the ingestion of plastics in different seabird species in order to evaluate their suitability as biomonitors of plastic litter in the BoB. For that purpose, dead seabirds were obtained, in both the northern and southern sectors of the BoB, from beach surveys, from recovery centres and from a Natural Reserve. A total of 120 seabirds of seven species were analysed: Common Guillemot (*Uria aalge*; n = 60), Razorbill (*Alca torda*; n = 10), Atlantic Puffin (*Fratercula arctica*; n = 11), Atlantic Gannet (*Morus bassanus*; n = 23), Black-legged Kittiwake (*Rissa tridactyla*; n = 8), European Herring Gull (*Larus argentatus*; n = 6) and Northern Fulmar (*Fulmarus glacialis*; n = 2). Except for the Razorbill, plastics were found in all the studied species. Species showing the highest prevalence of plastics (% of birds with at least one plastic) was the Northern Fulmar (100% of individuals), followed by the Atlantic Puffin (27%) and the Kittiwake (25%). The prevalence in other species was between 10 and 20%, even whether spatial differences in species prevalence was observed between the northern and southern sectors. The potential use of these species as biomonitors to assess plastic pollution and its contribution to the implementation of the MSFD in the BoB will be discussed.

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Keywords: plastics, seabirds, Bay of Biscay, Marine Strategy Framework Directive

## Double trouble in the South Pacific subtropical gyre: Increased plastic ingestion by fish in the oceanic accumulation zone

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Marine plastic pollution has received much attention from academia and media in the recent years, especially in relation to plastic ingestion by marine organisms. Fish are an important food source for island countries, yet there is little information on plastic ingestion by commercial marine fish species in the South Pacific (SP) region. In this field study, 932 specimens belonging to 34 commercial fish species from four SP locations (Auckland, Samoa, Tahiti and Rapa Nui), and some of their prey, were examined for plastic ingestion. The average sample size was  $N = 25.2 \pm 1.3$  specimens per species. Analytical methods for the isolation of plastic from the gut content included chemical digestion of the organic portion of the gut content, subsequent vacuum filtration on a set of stainless steel filters with the minimum mesh size of 63  $\mu\text{m}$ , and microscopic analysis of the filters. Plastic debris, mainly microplastics ( $\sim 95\%$ ), was found in 33 species. Across all locations, a total of 550 plastic particles was recovered from 226 specimens, which is an average ingestion rate (IR) of 24.3 % and a plastic load (PL) of 2.4 particles per fish. Rapa Nui fish exhibited the greatest IR (50.0 %), which were significantly greater ( $p < 0.05$ ) than IR from the other locations. Rapa Nui is located within the SP subtropical gyre, with low primary productivity and high concentrations of plastic debris. Thus, we suggest that these two factors, greater availability of plastic and decreased food availability, act together to cause significantly greater ingestion rates in Rapa Nui. Plastic was also found in prey from benthic and pelagic predatory fish, which confirms the trophic transfer of microplastics. Further research is needed into the occurrence of marine plastics in seafood, as it has recently raised much concern over potential consequences for human health.

Keywords: Keywords: marine plastic pollution, microplastics, Easter Island, seafood contamination, synthetic polymer, trophic transfer.

\*Speaker

# Ingestion of plastic by fish: a comparison of Thames Estuary and Firth of Clyde populations

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This study compared plastic ingestion between pelagic and benthic fish populations from two UK watersheds: the Thames Estuary and the Firth of Clyde. The alimentary canals of 876 individuals were examined. Of twenty-one estuarine species investigated, fourteen ingested plastics, including predator (fish) and prey (shrimp) species. Overall, 32% of organisms ingested plastic, mostly fibres (88% of total plastics). More flatfish (38%) ingested plastics than other benthic species (17%). In the Thames, more plastic was ingested by pelagic species (average number of plastic pieces ingested: 3.2) and flatfish (average number of plastic pieces ingested: 2.9) than by shrimp (average number of plastic pieces ingested: 1). More fish from the Clyde ingested plastic than similar Thames species (39% compared to 28% respectively); however, the average amount of plastic ingested did not differ between the sites.

Keywords: microplastics, microfibrils, fish, Thames Estuary, Firth of Clyde, shrimp, Crangon crangon, Platichthys flesus

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# Microplastic monitoring in the Orkney Islands, Scotland

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Microplastic contamination of UK coastlines is frequently associated with heavily urbanised and industrialised areas. However, remote locations such as the Orkney Islands, Scotland appear to be equally as contaminated, likely due to complex tidal patterns redistributing marine litter. This study examines the susceptibility of local fauna across both benthic and pelagic realms. Green shore crabs (*Carcinus maenas*) are the third largest fishery in Orkney. Microplastics were observed in the guts of 98.7% of individuals (n=77), predominantly fibres (74%) across nine locations in Orkney and one

in Shetland (Fig. 1). Whilst jellyfish are not fished in Orkney waters, fisheries in 15 countries do exist. Lions mane jellyfish (*Cyanea capillata*) (n=46) were collected in Orkney and Hoy with 952 plastic pieces (88% microfibrils) recovered, whilst *C. lamarckii* (n=32) yielded 323 pieces (81% microfibrils). Microplastics were also present in all water samples (n=340) from the same sites with 1,495 pieces of plastic recovered (93% microfibrils), with black and blue fibres most commonly observed. Orkney is also an internationally important breeding site for harbour (or common) (*Phoca vitulina vitulina*) seals, however an ~ 80% decline in population was observed between 2000-2013. Whilst microplastics are not thought to be a direct factor, concerns have been raised regarding impacts of microplastic ingestion on overall general fitness of seals. Seal fish prey (n=123 from nine species) are currently being examined to determine microplastic loading in the gut (n=101) and gills (n=109) from five locations around Orkney. This study will help to build a profile of biota contaminated by microplastics in this remote region with potential implications for fisheries and conservation.

Keywords: Green shore crabs, Cnidaria, Ctenophores, Seals, Fish, ingestion, trophic transfer

\*Speaker

## DISTRIBUTION AND BIOLOGICAL EFFECTS OF MICROPLASTICS IN MARINE ORGANISMS FROM THE ADRIATIC SEA

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Microplastics represent a growing concern for marine organisms due to their ingestion at all trophic levels, and the onset of potentially adverse effects. The distribution of microplastics along a typical marine food web was assessed in representative invertebrate and vertebrate species from the Northern, Central and Southern Adriatic Basin (Mediterranean Sea). Microplastics' size, shape and polymer typology were characterized by microscopy and  $\mu$ FTIR technology. Analyses revealed that microplastic ingestion is widespread, with the occurrence of at least one item in approximately 25% of the 500 analyzed specimens. Higher frequencies were observed in organisms of Central and Southern areas than in the Northern Adriatic, suggesting the influence of the different bathymetry,



morphology and main currents circulation: instead, no clear relationship could be observed between microplastics ingestion and trophic position, feeding strategy or habitat preference of analysed species. Extracted particles exhibited geographical differences also in terms of size, form and polymer typology with polyethylene, polypropylene and polystyrene representing the most abundant polymers.

The ecotoxicological effects of microplastics and their role as vehicles of PAHs were further investigated in the Mediterranean mussels, *Mytilus galloprovincialis*, exposed to polyethylene microparticles, both virgin and pre-contaminated with benzo(a)pyrene. After ingestion, microplastics were observed in different tissues causing a significant bioaccumulation of B(a)P. Analyses of molecular and cellular biomarkers highlighted the high susceptibility of the immunological parameters, while minor effects of treatment typology and time of exposure were measured for antioxidant system, lipid peroxidation, neurotoxic and genotoxic responses. The marked accumulation of B(a)P, the downregulation of the immune system and the increased toxicity observed in mussels when exposed to contaminated particles, suggest that exposure to microplastics might have long-term consequences on organisms health status. Overall, this study provided new insights on the distribution of microplastics in marine food webs, and on the mechanisms for their potential toxicity.

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Keywords: Microplastics, ecotoxicological effects, distribution, marine organisms,  $\mu$ FTIR

## Long-term community effects of nano- and microplastics on freshwater benthic macroinvertebrates

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## From Science to Solutions: Investigating the Impacts of Marine Litter on the Galapagos Marine Foodweb

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Marine plastic litter is a global conservation issue of high concern. The Galapagos Archipelago is no exception. Marine litter pollutes numerous locations around the Islands, although its sources, distribution and effects remain unstudied. Galapagos is a unique habitat: its geographical isolation, climatic conditions governed by the convergence of several great ocean currents and El Niño events and varied habitats give rise to endemic species found nowhere else on Earth. Many have evolved to fill specific ecological niches and their ability to adapt to rapid environmental change is unknown – baseline data from indicator species at different levels of the food chain is necessary to understand population level effects of threats (Denkinger & Vineuza, 2014). Working with the Galapagos Conservation Trust, Galapagos Science Center, the University of Exeter and a wide partnership network, we are beginning to fill key knowledge gaps around the sources and sinks of plastics in the Galapagos environment, the human behaviours that affect plastic pollution and ecological impacts. Until now, data on the impacts of marine plastics on wildlife in Galapagos has been opportunistic and mostly focused on entanglement risk. This project aims to investigate the impacts of marine litter and associated contaminants in the marine foodweb with a focus on marine invertebrates. In this presentation, I will provide initial results from our first field campaign to establish microplastic presence and compartmenting in different habitats in the Galapagos Marine Reserve and their presence in selected marine invertebrate communities. I will also present the methodology and first results from our citizen science beach and street litter surveys undertaken with local community groups to demonstrate the multi-disciplinary approach to the project.

Keywords: microplastics, Galapagos, marine litter, trophic, foodweb, citizen science

\*Speaker

## Microplastics in small coastal and off-shore fishes in the Baltic Sea

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We studied microplastic (< 5 mm) ingestion by small coastal and off-shore fish in the northern Baltic Sea. Coastal fish caught with a beach seine at nine locations and off-shore fish caught by trawling were collected from the different sub-basins of the northern Baltic Sea. The contents of

the gastrointestinal tract of 503 coastal and 673 off-shore fish individuals were analysed after tissue digestion for the enumeration of ingested microplastics. In coastal areas microplastics were found in 8 % of the studied fish individuals and in off-shore areas in 0.6% of all fish individuals studied. There were differences between the studied locations and the prevalence of microplastics was higher in coastal fishes. No relationship was found between the stomach fullness of fishes and plastic ingested. Nor was there a correlation between the frequency of microplastic ingestion by fish and the amount of microplastics in seawater.

Keywords: Microplastics, northern Baltic Sea, coastal vs. offshore fish

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## **November 21st – Panel 13.3: 16h30 - 18h30 (AGH), Panel chaired by Andy Booth.**

### **A standard protocol for the assessment of microplastics toxicity using planktonic marine organisms**

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Oceans are the final sink of land-based plastic litter that fragments into microplastics (MP), available for incorporation into oceanic trophic webs through ingestion by zooplankton. A standard protocol was developed to test the toxicity of MP using early life stages of marine invertebrates, including the *Paracentrotus lividus* sea urchin. The protocol includes a leachate test and a particulate test. The leachate test is conducted with particles < 250 µm while the particle test is conducted with the size range < 20 µm in order to allow ingestion of the particles by the test organisms. Therefore, the toxicity of chemical additives, sorbed chemicals and particles themselves are taken into account. MP are kept in suspension by using a rotatory wheel at 1 rpm. Leachates are made up using a 10:1 liquid to solid ratio, as recommended in standard solid-waste leachate toxicity testing. Particles are tested at concentrations up to 100 mg/L. This is intended to rank MP according to their toxicity and not to represent environmental values. When necessary, testing materials are grinded using an ultracentrifuge mill after embrittlement with liquid N<sub>2</sub>. Particle size distribution is recorded using a Multisizer 3 Coulter Counter. Results support lack of toxicity of virgin polymers, including polyethylene and PVC, and instances of toxicity for MP obtained from consumer products made of the same polymers. Therefore, additives are the most likely cause of the toxicity found. Metals used as processing aids were measured in the leachates at concentrations above toxicity thresholds for the testing organisms. Concerning the particle tests, levels found to be toxic are many orders of magnitude above those reported even in the most polluted oceanic waters.

Keywords: microplastics, toxicity, larval bioassays, sea, urchin, zooplankton

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# ADSORPTION OF MERCURY TO MICROPLASTICS AND VECTORIZATION TO MARINE ORGANISMS

Juan Santos-Echeandía \*† 1, Beatriz Fernández 1, José Roberto Rivera-Hernandez 1,  
Juan Bellas 1, Soledad Garrido 1, Leticia Vidal-Liñán 1, Diego Rial 1, Concepción  
Martínez-Gómez 1, Marina Albentosa 1

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One of the most concerning aspects of microplastics (MPs) in marine habitats is that they might act as vectors of pollutants to marine organisms. The research about the sorption of metallic contaminants by MPs in the marine environment is limited. This is related to the fact that MPs are generally regarded to be relatively inert towards aqueous metals ions, contrarily to what occurs with organic contaminants. However, among the IEO's activities in the EPHEMARE project, an adsorption of Hg to microplastics has been demonstrated and the following activities have been carried out:

WP1: The adsorption and desorption kinetics of Hg to virgin microplastics (high density polyethylene, 8 µm) have been evaluated. In addition, a comparison with the adsorption/desorption kinetics of this metal to microalgae (*Isochrysis galbana*, t-ISO) has been checked.

WP2: A laboratory experiment to evaluate the uptake and elimination rates of Hg adsorbed to different surfaces (MP, MA) compared to dissolved Hg by adult mussels with time was carried out. Mussels were selected as model filter-feeder invertebrates. In addition, the accumulation of this metal in different tissues with time upon the different treatments was monitored.

The results obtained in these experiments will be presented in the congress. WP3: The toxicity of Hg-charged microplastics was evaluated in mussels and sea-urchin embryos, measuring the mortality rates and growth with respect to the control or natural conditions. In vitro bioassays with hemocytes were used to evaluate the immune responses to Hg-charged microplastics.

*Keywords: mercury, microplastics, mussels, seaurchin, toxicity*

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## Are microplastics facilitating the accumulation of organic pollutants in benthic bivalves?

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Microplastics are potential vehicles of other contaminants in the marine environment to food chains. For this reason it is important to understand both the impact of microplastics alone as well as from those with adsorbed chemicals in marine organisms. In this sense the work presented here investigates the effects of microplastics in the benthic marine bivalve *Scrobicularia plana* after 14 days exposure to 1 mg L<sup>-1</sup> to polystyrene (PS; 20 µm spheres), and of low-density polyethylene (LDPE) of different size ranges (4-6, 11-13 and 20-25 µm particles) with and without adsorbed persistent organic contaminants: benzo-a-pyrene (BaP), perfluorooctane sulfonic acid (PFOS) and benzophenone-3 (BP3). Clams were sampled at the beginning of the experiment (day 0) and after 3, 7, and 14 days of exposure. A multibiomarkers approach was used to investigate the effect of exposure in different clam tissues, gills, digestive gland, and haemolymph. Antioxidant (superoxide dismutase, catalase, glutathione peroxidase) and biotransformation (glutathione-S-transferases) enzyme activities, oxidative damage (lipid peroxidation levels), genotoxicity (single and double strand DNA breaks), and neurotoxicity (acetylcholinesterase activity) were assessed. PS accumulated in tissues and induced genotoxicity with time. BaP adsorbed in LDPE was accumulated in clam tissues. Oxidative stress was noted in gills for all LDPE+adsorbed chemicals. An increase of biotransformation enzymatic activity was noted in gill tissues for all MP treatments over time. Neurotoxicity effects were observed after exposure to LDPE+BaP and LDPE+BP3. Oxidative damage was also observed in all LDPE+adsorbed contaminants. Some evidence suggests that LDPE+BP3 and LDPE+BaP induces genotoxicity over time. Overall results showed a tissue-specific biomarkers response with gills being more affected by exposure to microplastics than digestive gland and biomarkers alterations apparently more related to the toxicity of adsorbed chemicals than to microplastics alone.

\*Speaker. Keywords: microplastics, bivalves, BaP, BP3, PFOS, LDPE, PS

# Microplastics as vectors of organic contaminants in the marine environment

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One of the most concerning aspects of microplastics (MP) in marine habitats is that they might act as vectors of pollutants to marine organisms, since hydrophobic organic contaminants (HOCs) with low water solubility tend to concentrate on the surface of these particles. In a similar way, HOCs have also a high affinity for particulate organic matter, such as microalgae cells. Both MP and microalgae may also release these compounds, acting as vectors of pollutants to marine organisms. HOCs bioaccumulation and associated toxicological effects might be dependent on the nature of the particle through which the chemical is transported. IEO activities in the EPHEMARE project included:

WP1: MP were loaded with organic pollutants: chlorpyrifos (CPF) oxybenzone (BP3), fluoranthene (FLUO) and phenanthrene (PHE). A high density polyethylene powder of small particle size, close to the size of the microalgae (ca. 8  $\mu\text{m}$ ), was selected. The partition of the pollutants between the particulate and aqueous phases was evaluated.

WP2: MP uptake and elimination were studied using mussels as a filter-feeder model organism. Kinetics were studied with virgin MP and with MP loaded with CPF. The affinity of CPF for MP was compared with another adsorption surface present in the water column: phytoplankton. The accumulation of CPF in mussel tissues was carried out with both types of particles.

WP3: The toxicity assessment of virgin and loaded MP at the organism level was performed using embryos and larvae of sea-urchins and mussels, and with microalgae. Biological responses were: the percentage of normal larvae (mussels), the larval growth (sea-urchin) and the growth rates (microalgae), with respect to controls.

WP4: The toxicity of MPs loaded with CPF was evaluated using a battery of biomarkers that comprise different levels of organization: individual (Scope for Growth, SFG), cell (immune response) and metabolism (oxidative stress and acetylcholinesterase).

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Keywords: microplastics, impact, Hydrophobic organic compounds, toxicity, marine invertebrates, phytoplankton

## Plastics debris as organic pollutants vector between littoral areas and the sea

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Plastics act as passive samplers in the environment, accumulating hydrophobic organic contaminants which are present in the surrounding compartments (air, water, soil, seawater, etc.). Then plastic polymers concentrate hydrophobic organic contaminants by sorption or specific interactions, which can be transported to other systems such as the marine environment through direct discharges and transport from coastal areas (air, rivers, etc) or from sea to beaches. In this study plastic debris not previously immersed in seawater were sampled in the surrounding area of a Mediterranean lagoon in order to determine the concentration of 91 persistent and emerging organic contaminants (PAHs, current-use pesticides CUPs, personal care products- PCPs and plastic additives). Their desorption was characterized for the first 24 h from different polymers to seawater and the remaining content of these contaminants was also extracted by ultrasonic extraction with methanol. A significant fraction of sorbed contaminants in polymers was desorbed in the first 24 h, particularly for triazines and organophosphorus pesticides due to their lower hydrophobicity than other considered analytes. The remaining contaminants contained in plastics can be also transferred to seawater, sediments or biota. Additionally plastic and microplastics were sampled from 3 Mediterranean beaches to evaluate their concentrations of organic contaminants and the transfer of contaminants from sea to coastal areas. PAHs, CUPs and PCPs were found in the plastic extracts from all studied areas and their concentrations were related to predominant anthropogenic activities (touristic, agriculture, urban, etc). The most abundant contaminants in plastics were PAHs and PCPs showing the relevance of transport, urban and touristic activities as pollutant sources in the three studied coastal areas. The present study demonstrates plastics act as passive samplers in the environment accumulating hydrophobic organic contaminants from air, seawater and particulate matter and play a role as pollutant transport vector from continental areas to sea and viceversa.

Keywords: plastic debris, desorption, organic pollutants, seawater, contaminants of emerging concern, transfer

\*Speaker



# Comparison of sorption capacity of persistent organic pollutants (POPs) on plastic resin pellets from different origins using a quick sorption method

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According to A European Strategy for Plastics in a Circular Economy, in 2015 the EU generated 26 million tons (Mt) of plastic post-consumer waste. 1.75 Mt of 7.8 Mt collected for recycling was exported to China. China's ban on waste import starting from 2018 forces EU to take sustainable alternatives. Reduction/prevention of the use of synthetic plastic products will be the must. It is likely that the use and the variety of biodegradable and recycled plastic materials is to be increased. This shift in plastic waste management also requires the study to investigate sorption behaviours of pollutants onto new plastic materials. Here we present a quick and reproducible method to compare sorption capacity of persistent organic pollutants (POPs) on a variety of plastic resin pellets from different origin. Resin pellets tested here were made from widely used synthetic polymers, post-industry and post-consumer recycled products as well as biodegradable polymers.

The sorption test was conducted in water containing selected POPs with cosolvent (water: solvent = 70:30, volumetric). Cosolvents selected were miscible in water, owns a vapour pressure smaller than 60 hPa and can dissolve selected POPs in mg/L range. Sorption capacities of the POPs on pellets from different origins were compared by exposing a given mass of pellets in the mix-solution containing the POPs with a fixed starting concentration, 1000 µg/L. In addition, partition coefficients of selected POPs between the solution and pellets from different origin were determined by varying the initial POP concentrations in the mix-solution. The derived partition coefficients were evaluated whether they can be used as predictors to derive partition coefficients of the POPs between water and the polymers. TU Darmstadt and CARAT are participants of an EU project "PLASTOX", a consortium of a JPI Oceans' Joint Action. TU Darmstadt is funded by BMBF.

Keywords: microplastics, resin pellets, quick sorption method, persistent organic pollutants, recycled, biodegradable

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# Effects of Leachates from Weathered Microplastic in Cell-Based Bioassays

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The toxicological effects of microplastic (MP) are often studied in the laboratory using pristine particles, which may be of limited environmental relevance since plastic in the environment is weathered by UV light irradiation, mechanical stress, salinity and other factors. We applied cell-based bioassays to study the effects of chemical mixtures that leached from the most common polymers in European commerce (largely additive-free, pre-production pellets of PE, PET, PP, PS) and positive controls (e-waste and a new keyboard with high content of additives) after weathering under intense UV light in artificial seawater. All test materials were processed with corresponding dark controls. The chemicals in the seawater leachates were enriched by solid-phase extraction. The concentrated aqueous leachates were then dosed into the bioassays, covering i) cytotoxicity; ii) activation of metabolic enzymes via binding to the arylhydrocarbon receptor and the peroxisome proliferator-activated receptor (PPAR $\gamma$ ); iii) specific, receptor-mediated effects (estrogenicity); and iv) adaptive stress response (oxidative stress). Further, non-target analysis using LC-HRMS was used for tentative identification of compounds liberated from the test material during weathering. The positive controls showed high activity in all assays and thus provide a proof-of-concept that the experimental setup can demonstrate effects of the chemicals liberated during weathering. Only oxidative stress response was significantly higher in the leachates from the pre-production pellets than the blank response for all polymers, while the other bioassays did not elicit significant responses. In a few cases, the UV treatments showed higher effects than the corresponding dark controls, e.g. PE in PPAR $\gamma$  or PP for oxidative stress. Our results give insight into possible effects of the mixtures of chemicals in leachates from weathered plastic.

Keywords: weathering, degradation, plastic, aquatic environment

\*Speaker

**November 22nd**

**November 22nd – Panel 15.1: 8h30 - 10h (AGH),  
Panel chaired by TBD**

## Trace nanoplastic and microplastic fiber analysis in wastewaters and activated sludge: synthesis and utility of metal doped plastics

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Research on particulate plastic and their distribution and effects in the environment has intensified in recent years; but truly quantitative analysis has remained elusive in part due to analytical difficulties. Synthesizing plastic materials with a metallic, chemically entrapped tracer provides a robust way to more easily, accurately and quantitatively detect particulate plastic in complex environmental and biological media. In this work, we synthesized a variety of particulate plastics (nanoplastic particles, fibers) which encompass a suite of various sizes and dimensions, surface morphologies (smooth, rough) and polymers (polystyrene, polyester). Each variant has an embedded metallic fingerprint (Pd or In; approx. 0.5% metal/wt) which can be used to detect plastic by analytical techniques for metals analysis, such as ICP-MS and TEM/EDX. This allows us to more quantitatively and quickly assess plastic in complex matrices at particle number concentrations orders of magnitude lower and (much) smaller particle sizes than is currently possible with other analytical techniques. To highlight the utility of this approach, we investigated the attachment efficiency of particulate plastic to sludge flocs (which occurs in < 30min) and determined plastic removal in batch experiments representing the activated sludge process a municipal wastewater treatment plant (WWTP). With a recovery rate of plastics over 95% in all experimental sets, we found over 98% of plastics (both nanoplastic particles and microplastic fibers) in the sludge, with a high correlation between TSS concentration and plastic concentration. Beyond the case study specifically highlighted here, these metal laden plastics are suitable to study fate, transport, ecotoxicity and interactions with organisms at trace concentrations. By using these materials, bench

scale and pilot scale studies can be used as a bridge to understand the environmental processes that dominate (particulate) plastic fate, transport and interactions with biota until analytical techniques to measure native plastics at trace concentrations have matured.

\*Speaker

Keywords: Nanoplastic, Textile Fiber, Microplastic Fiber, Wastewater Treatment Plant

# A NOVEL MICROPLASTIC EXTRACTION TECHNIQUE FOR THE DETECTION OF MICROPLASTICS IN THE LIVER, BLOOD AND FILLET OF EUROPEAN SEA BASS, DICENTRARCHUS LABRAX

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There is a significant increase in plastic waste accumulation in marine systems. Microplastics (MP) have been already detected in the muscular tissue or filet of mussels but are not yet investigated in the case of more commercially significant fish species. Marine nutrients used for human diets represent a particular problem, since MP is also absorbed by humans. Therefore, the overall aim of this project was to develop a suitable method for the detection of MP in the tissue of fish and to study the translocation of MP from the feed to the tissue of fish. This is the first time the novel developed extraction method has been used in routine food monitoring. A 16- week controlled feeding experiment with juvenile sea bass (*Dicentrarchus labrax*) was conducted with 120 fish per tank in quadruplicate. Fish were fed ad libitum with a control diet (without MP additive) and a test diet (with defined amount of fluorescent MP particles- ~ 1-5 µm) under controlled conditions in a recirculation system. Growth was monitored at experiment onset and termination. Liver, blood and muscle tissues of fish (filet) were taken for maceration, digestion and subsequent determination of MP content. Detection of MP in sea bass fillet from experiment will be detected with fluorescence microscopy. In the case of commercial sea bass fillet: from wild and aquaculture product from market, detection of MP will be carried out with FT-IR microscopy and Raman microscopy. Final results are pending! But we expect that the extraction method being developed for the detection of MP will be new to the industry, and can be implemented as a standardized method for MP detection and quality control in commercial fisheries.

Keywords: Microplastic, translocation, KOH, fish, sea bass, liver, blood, fillet

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## New methodologies to collect and analyse micro-debris ingested by loggerhead turtle (*Caretta caretta*).

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The European Project INDICIT had proposed sea turtles as marine debris indicator on the European waters. One of the main objectives was to homogenize and standardize methodologies to collect and analyse macro and micro-debris ingested by sea turtles, on dead and alive turtles. In order to determinate microplastic ingestion by sea turtles, INDICIT project had standardised methodologies for sampling, where the minimum debris size was established in 1mm to avoid contamination. On dead turtles, necropsies were carried out and complete digestive track was analysed by sections (oesophagus, stomach and intestine). Digestive content of each section were collected and analysed.

For live animals, stranded turtles arrived to the recovery centres were sampled. Each turtle were placed at individual tanks, feed with eviscerated food to avoid contamination, and water tank sampled for one month. Faeces were filtered with 1mm mesh sieve daily during the first ten days and 3 times per week up to one month after arriving date.

The new methodology developed by INDICIT consisted on filtering the digestive content obtained from the necropsies, or the faeces filtered from individual tanks hosting live animals, through 2 sieves train (5mm and 1mm mesh) to separate macro and micro-debris samples. Micro-debris sample were digested with H<sub>2</sub>O<sub>2</sub> or KOH to eliminate organic material and obtain a clean sample with only non-organic material. Preliminary results conducted on sea turtles (dead and alive) stranded in Canary Islands showed 100% of microplastics occurrence, perhaps induced by the greater accuracy of the new methodology. The "2-sieve method" could be tested on other taxa, to verify if is an adequate methodology to analyse micro-plastic ingestion on biota.

Keywords: Turtles, *Caretta caretta*, Canary Islands

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# Comparison of chemical extraction and microwave-assisted digestion techniques for metal desorption from plastic pellets by ICP-MS

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Both primary and secondary microplastics contain chemical additives from their manufacturing process and are known to accumulate organic and inorganic contaminants, including metals, from the surrounding environment. There is now evidence that the ingestion of these microplastics by aquatic organisms can lead to increased exposure to contaminants that are potentially transferred up the food chain. While previous studies have investigated the desorption of metallic contaminants from polymers, no standardised extraction methodology exists in the literature and therefore many different chemical extraction processes have been used. The aim of this study was to evaluate five different chemical extraction methods and a microwave-assisted digestion technique (commonly used in environmental analytical procedures) for the desorption of metals from virgin plastic pellets in a laboratory-based study. Pellets of two virgin polymers, polyvinyl chloride (PVC) and polyethylene (PE) were agitated for 100 hours in a known concentration of metals and water in LDPE bottles. Pellets were removed from the metal solution and individually placed in eppendorfs and dried under a fume hood. Triplicate samples of the metal solution from the initial and final exposure time were preserved with 1% HNO<sub>3</sub>. To determine if there was a transfer of metals to the walls of the LDPE bottles, the bottles were agitated for 24 hours with 10% HNO<sub>3</sub> and samples were preserved for future analyses. Four desorption extractions were performed for 24 hours under agitation with 3 mL of 10% HNO<sub>3</sub>, 10% HCl, NH<sub>4</sub>OAc-EDTA and 20% Aqua regia. A milli-Q extraction was also performed as a control. In all cases, triplicate samples were analysed. In addition to the extractions, two digestions with 10 mL (8+2) of HNO<sub>3</sub> + H<sub>2</sub>O<sub>2</sub> and HNO<sub>3</sub> + H<sub>2</sub>SO<sub>4</sub> were carried out to identify metal concentration in each pellet. Elemental concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS).

Keywords: adsorption, desorption, metals, methodology, ICP MS

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# Fingerprinting of chemicals released from plastic polymers by UV-light

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Plastics in the marine environment are degraded to free chemicals due to radical reactions initiated by sunlight. The goal of this study was twofold, first to identify chemicals released by different plastic polymers under UV-light exposure and second to establish fingerprints of the mixture of chemicals released by the plastics to identify different plastic polymers in unknown samples. In the first part of this study we developed a "weathering wheel" in which plastic particles floating in water were exposed to UV-light (see graphical abstract). We exposed pre- production pellets of PE, PP, PS and PET for five days, which corresponds to approximately 510 days of sunlight exposure to European mean solar irradiance. The chemicals released into the water were subsequently concentrated using solid phase extraction (Oasis HLB Plus, Waters) and analysed by liquid chromatography coupled to high resolution mass spectrometry (LC-Orbitrap Q Exactive HF, Thermo). By using non-target and suspect-screening methods we were able to identify homologues series of low-molecular weight polymer fragments with oxidized end groups. 22 polymer degradation products were identified at high levels of confidence, mostly dicarboxylic acids. In the second part of this study, we mimicked environmental samples by exposing six different plastic polymers (PE, PP, PS, PET, PU and PVC) to UV-light in the presence of sand and water. After exposure, extraction and analysis not only the identified chemicals from the first part of this study but also the full spectrum of detected peaks can be used to establish a "fingerprint" for each polymer. We examine the hypothesis that these fingerprints can be used to identify the presence and composition of mixtures of plastic polymers in unknown samples.

Keywords: weathering, UV, light, free chemicals, fingerprinting

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## **November 22nd – Panel 15.2: 8h30 - 10h (AGH), Panel chaired by TBD**

### **Inter-annual monitoring of microplastics in marine intertidal sediments of the Firth of Forth**

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Pauline Sechet 1, Rachel Wood 1, Neil Mearns 1, Shanna Paterson 1, Mégane  
Viguiard 1, Holly Walker 1, Fergus Kinsley-Willis 1, J Decclan McCreton 1

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Microplastics (MP), defined as pieces of plastic < 5mm are commonly found in the marine environment and originate either from consumer care products and plastic production plants or from the disintegration of larger pieces. MPs need to be monitored in order to evaluate the effectiveness of Government initiatives to reduce plastic debris in the environment. The aim of the present study, therefore, was to contribute to the development of a hitherto lacking quantitative long-term marine MP database. We present the results of a three-year pilot project in the Firth of Forth, point to innovations in sampling and contamination prevention, as well as the limitations. Sediment samples were obtained in triplicate from intertidal sites in May2014, May & Sept2015, May & Sept2016, 2017 using glass bijoux tubes as miniature cores and sealed with metal screw caps, processed using a density separation procedure and the polymer types determined using FT-IR spectroscopy. Results: plastic particles (34-4,800 kg-1) and fibres (1,700-4,300 kg-1) along both shores of the Firth of Forth. The number of Fibres was generally higher than particles, with no apparent pattern of spatial distribution. Although a spike in MP particles was observed in Sept2015 and May2016, there was no significant difference in MP particle concentrations between May 2014 and May 2017. There was also no significant difference in MP fibre concentrations during the same three-year period and no evidence of seasonal fluctuations. MP concentrations in intertidal sediments in the Firth of Forth have remained stable. This is significant baseline information and will be instrumental in assessing the effectiveness of Government policy regulating the use of particularly single-use plastic products. However, in order to compare results between countries and laboratories, for the purpose of gaining a more global insight into the microplastics contamination issue, more standardized sampling and extraction procedures need to be developed.

Keywords: microplastic, fibres, particles, intertidal sediments, monitoring, baseline



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# THE CONTRIBUTION OF AQUACULTURE INDUSTRY IN THE GLOBAL MARINE MICROPLASTICS POLLUTION: FIRST RECORD OF LEVELS AND DISTRIBUTION IN COASTAL SEDIMENTS COLLECTED NEAR A PRODUCTION SITE IN THE SOUTH WEST OF NORWAY

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Elisa Ravagnan , Maj Arnberg

*1 NORCE – Norway*

Plastic waste is of increasing concern in the aquatic environment. A large portion of the plastic waste is produced onshore and reaches the marine environment, which is considered the main sink of plastic debris. Floating plastic particles accumulate in pelagic habitats. However, due to the biofilm formation they eventually sink and accumulate on the seafloor together with non-buoyant by design plastic particles posing risk to the benthic communities. Aquaculture makes wide use of durable plastics, which are used for tanks, fish cage collars, pond liners, netting, rope, and floats, among other items. In the context of global plastic pollution of the oceans, aquaculture is a tiny contributor, although estimation of the size of the contribution remains a knowledge gap and lost or derelict gear from aquaculture can be a locally important contributor. In the present study, a preliminary assessment of the occurrence, levels and chemical characterization of 6 type of polymers in sediments near an aquaculture located in the Boknafjorden (Norway) is presented. Plastic microparticles were extracted by flotation from 5 kg sediment samples, purified by a multi-step combined enzymatic and mild oxidant reaction and finally analysed by sequentially visual microscopic inspection and thermal desorption pyrolysis gas chromatography/mass spectrometry (GCMS-pyr). Most of the detected polymers were identified as polypropylene, polyethylene, polyvinylchloride followed by polyethylene terephthalate, polystyrene and polyamide. An attempt to identify and quantify plastic based paints and antifouling agents was performed. Preliminary results are presented

Keywords: Aquaculture Industry, Sediments, Microplastics, GCMS, pyrolysis

\*Speaker

## Fast and manageable determination of the microplastics in the sediment

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The main goal of this research was to obtain a method and approach for microplastics determination in the sediment that would take care of possible density changes due to biofouling; that could be used independently of the different upper size limit for microplastics; to have ease of use so that it can be applied on the expedition ship and would be inexpensive. Separation of microplastics from the sediment, based on the difference in densities is a well-known and adopted approach, due to a nominally lower density of microplastics than the density of sediment. The novelty of the proposed approach is the use of sodium polytungstate or SPT ( $\text{Na}_6[\text{H}_2\text{W}_{12}\text{O}_{40}]$ ), a non-toxic compound used as a heavy liquid for gravity separation. Due to its very high solubility in water, different density can be achieved, from 1.01 g/cm<sup>3</sup> to 3.10 g/cm<sup>3</sup>. Prior to separation procedure, samples should be treated with H<sub>2</sub>O<sub>2</sub> (30%) to remove organic matter and with HCl (10%) to remove carbonate particles. These steps are important because shells of some organisms and similar hollow objects remain in the lighter fraction and affect a determination of microplastics later on. A sample prepared in this way is then separated in the SPT solution (density 1.60 g/cm<sup>3</sup>). Microplastics particles float on top of the SPT solution and the remaining sediment sinks to the bottom. A solution is then frozen and the light fraction is easily separated. Extracted microplastics should be washed, dried and weighted, to calculate its amount in the sample. Final determination of extracted particles and verification of the type of microplastics is done by Fourier transform infrared spectroscopy (FTIR). A determination could be done by some other methods but proposed one has an advantage when a small amount of microplastics particles is extracted, e.g. 1 mg, by making KBr pellets.

Keywords: Microplastics, Sediment, SPT, FT, IR

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## Sediment trapping – A method for monitoring microplastic litter influx in aquatic sediments

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Microplastic litter has been reported from wide range of aquatic environments. Concentrations of up to thousands of particles per one kilogram of sediment have been reported from marine beach sediments as well as from lacustrine littoral sediments. Due to a lack of chronological control, previous studies do not enable evaluation of the influx rate of microplastic pollution, which is a crucial aspect for environmental monitoring. In order to confirm and compare the feasibility and efficiency of different environmental conservation methods applied in order to reduce microplastic contamination in aquatic environments, an accurate method for monitoring the pre-conservation influx as well as the changing influx during and after the acts of conservation, is needed. A sediment trap method is widely applied in aquatic sedimentary studies in order to understand sedimentation processes in a certain environment. We have used a near-bottom sediment trap method in Lake Haukivesi, central Finland, for measuring and quantifying the microplastic influx rate during one year. Near-bottom sediment traps with two collector tubes and known surface area, fixed one meter from the lake floor, collect all particles that are about to accumulate on the lake floor. Controlled temporal interval of trap maintenance enables calculation and determination of local microplastic influx rate i.e. number of accumulating particles per time per surface area. Combined with heavy liquid separation technique, a method commonly applied for microfossil separation from sedimentary material, and FTIR technique, sediment trapping shows promising results. Near-bottom sediment trapping can be used for long term monitoring in order to gain a deeper understanding of the microplastic sedimentation processes and for supervising that the defined target conditions are met.

Keywords: microplastic influx rate, monitoring, sedimentation

\*Corresponding author: [saitur@utu.fi](mailto:saitur@utu.fi) †Speaker

# The First Occurrence, Spatial Distribution and Characteristics of Microplastic Particles in Sediments from Banten Bay, Indonesia: Potential Impact for Benthic-Pelagic Coupling Food Web

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Microplastics (MPs) are recognized as an emerging issue worldwide, which includes Indonesia. Due to limited information regarding the data of microplastic pollution in Indonesian water, We conducted an initial investigation on the occurrence, spatial distribution, identification and potential ecological impact of MPs in the sediments from different locations in Banten Bay, a shallow and semi-enclosed bay located at the northwest coast of Java, Indonesia. The bay is under very high population pressure due to increasing coastal development in recent decade. This study showed that microplastic pollution is prevalent in Banten Bay, where all sediments from 25 stations contained microplastics of various size and shape. The most common shape and size were foams (38% of observed microplastics), and more than 50% were in size range between 500 and 1000 $\mu$ m. The mean concentration of MPs recovered in the sediment samples was  $267 \pm 98$  particles/kg dw sediment. The particles were found to be more highly distributed in stations with fine sediment grain size and located near the river mouth of the island than offshore, which suggest the impact of present MPs in sediment might be harmful to benthic community and potentially increase the magnitude into pelagic community through benthic-pelagic food web system. Moreover, the river effluent is suggested as a pathway for plastic pollution to the Banten Bay.

Keywords: Microplastics, Banten Bay, Benthic, Sediment, Floation methods

\*Speaker

# Combining hydrodynamic modelling-based sampling with FPA- $\mu$ FTIR-Imaging automated analysis: first evidence of microplastic pollution in the sediments of the Limfjord (Denmark)

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Microplastic (MP) pollution affects almost the totality of the water bodies, including transitional environments such as lagoons, salt marshes, and estuarine areas. In order to improve the knowledge on MP contamination in the Scandinavian Region, we conducted the first MP sediment monitoring survey of the Limfjord (North Jutland - Denmark), the largest Danish estuarine area (1500 km<sup>2</sup>). Its average depth is 4.9 m, with a freshwater average inflow of 2.7 km<sup>3</sup> y<sup>-1</sup> and a net flow of 6.8 km<sup>3</sup> y<sup>-1</sup> passing from west to east due to the wind and tidal impacts. The total catchment area (7600 km<sup>2</sup> - 70% agriculture, 5% urban areas, 20% wood-lands) includes Aalborg (~ 130000 inhabitants) and other smaller cities, harbors and shellfish farms along the shores (all potential MP sources). The sampling locations were selected taking into account the hydrodynamical conditions and considering the bed shear stress induced by the currents as the main parameter for sedimentation, erosion, and resuspension of materials. The shear stress was modeled with a calibrated non-steady hydrodynamic model (MIKE3FM), including both effects of time-varying boundary conditions, stratified conditions due to salinity gradients and wave-induced currents based on a spectral wave calculation. Sampling was hence conducted in areas with low bed shear stresses, where the light material is expected to settle (minor re-suspension). Moreover, sampling was conducted both up and downstream of the major city (Aalborg), WWTPs and riverine inputs. Samples were collected with a Van Veen grab, processed by flotation (ZnCl<sub>2</sub>) and multi-step sample clean-up (enzymatic treatment, catalyzed H<sub>2</sub>O<sub>2</sub> oxidation), then analyzed by FPA- $\mu$ FTIR-Imaging spectroscopy/MP auto-analysis (MPhunter). Preliminary results showed a diffused MP contamination (77 - 270 part. g<sup>-1</sup>), with the highest level measured close to Aalborg. The polymer composition showed mainly polyester (35%), polypropylene (13%), nylon (11%), acrylic paints (11%), PVC (9%), polyethylene (5%) and alkyd paints (4%).

Keywords: Modelling, FPA  $\mu$ FTIR Imaging, Microplastic, Sediment, Limfjord

\*Speaker

## **November 22nd – Panel 16.1: 10h30' - 12h30' (AGH), Panel chaired by François Galgani.**

### **Microplastics in the deep: an assessment from the Rockall Trough, Northeast Atlantic Ocean**

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Microplastics, small pieces of plastic < 5 mm in diameter, are found extensively in the natural environment and present numerous ecological threats. While the ultimate fate of marine microplastics are not well known, it is hypothesized that the deep sea is the final sink for this anthropogenic contaminant. Here we present a quantification and characterisation of microplastics ingested by three benthic macroinvertebrates (*Ophiomusium lymani*, *Hymenaster pellucidus* and *Colus jeffreysianus*) and in adjacent water, > 2200 m deep, in the Rockall Trough, Northeast Atlantic Ocean. Despite the relative remoteness of this location, microplastics were identified in deep-sea water at a concentration of 70.8 particles m<sup>-3</sup>, comparable to that in surface waters. All of these particles were monofilament fibres and the majority were identified as polyester. Of the invertebrates examined (n = 66) 48 % had ingested microplastics with quantities enumerated comparable to coastal species. Both monofilament fibres and fragments were present however fibres were most numerous (87 %). A total of nine different polymer types were identified, with polyester again being the most abundant, and of note was the presence of the buoyant polymer polyethylene. Significant interspecific differences were evident, but microplastic abundance was not found to relate to organism feeding mode nor organism mass. The isolated microplastics were visually highly degraded; surface areas, calculated using photogrammetric methods found the surface areas of deep-sea microplastics to be more than double that of pristine particles. Future work will examine microplastics within the sediment, thus assessing quantities in each environmental 'reservoir' and the potential for microplastic sequestration by one of the largest global ecosystems. Additionally, microplastic abundance will be examined over an invertebrate time-series sampled from this permanent monitoring site to examine long-term temporal trends.

Keywords: deep sea, ingestion, long term fate, benthic

\*Speaker

# The imprint of microplastics from textiles in southern European deep seas

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Microfibers are among the most prevalent type of microplastics observed in the marine environment. They are mostly shed from synthetic textiles during production or washing, and may be entering the ocean via wastewaters and atmospheric fallout. Microfibers have been found in surface waters, sea ice, and in coastal and deep water sediments. The recent discovery of microfibre ingestion by deep-sea organisms in a natural setting has underlined the need to quantify of this human waste in the deep marine environment. Here we present new data on the distribution of plastic microfibers after a widespread survey of seabed sediments in southern European seas including the northeast Atlantic Ocean (Cantabrian Sea), the Mediterranean Sea (Alboran Sea, Catalan Sea, Cretan Sea and Levantine Sea) and the Black Sea at depths from 42 m at the continental shelf to 3,500 m in the abyssal plain. Such a wide depth range allowed investigating patterns of microfibers distribution along the coastal-deep sea continuum and understand their long-range transport. We report abundances of 10-70 microfibers in 50 ml of sediment, including both natural and regenerated cellulose, and synthetic plastic (polyester, acrylic, polyamide, polyethylene, and polypropylene) fibers. Following a shelf-slope-deep basin continuum approach, it would appear that coastal seas retain around 33% of the sea floor microfibers, but greater quantities of the fibres are exported to the open sea, where they accumulate in sediments. Submarine canyons act as preferential conduits for downslope transport of microfibers, with 29% of the seafloor microfibers compared to 18% found on the open slope. Around 20% of the microfibres found had accumulated in the deep open sea beyond 2000m of water depth. The persistent nature of microfibers accumulated in the remote deep sea makes evident the need to design effective management strategies for reducing emissions to the environment.

Keywords: microfibers, textiles, deep sea, Mediterranean sea

\*Speaker

# Microplastic occurrence and distribution from discharge points to deep basins in an urban model fjord

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Urban harbors are expected to be highly contaminated by microplastics from anthropogenic activities, wastewater, run-off and maritime traffic. Knowledge about relevant microplastic concentrations is vital for risk assessments, but the concentration ranges in marine environments are largely unknown. Uni Research has done marine monitoring since the 70's in the fjords around Bergen. The in-depth knowledge about abiotic and biotic factors in the fjord system makes it ideal for investigating levels of marine microplastics from urban sources. This study used sediment from discharge points to deep sedimentation sites in the Byfjorden in Bergen, Norway and extracted microplastic particles by density separation with Zinc Chloride in a Microplastic Sediment Separator. Polymers and particles (> 10 µm) were identified using Fourier Transform Infrared Spectroscopy at the Biologische Anstalt Helgoland of the Alfred Wegener Institute. The highest number of plastic particles (10-500 µm) was ~ 200000 kg<sup>-1</sup> dry sediment at the discharge site (KVR1). Distribution and deposition at stations ~ 320 m deep, followed expected patterns for organic deposits. The number of particles at deposition sites varied from 12000 to 71000 kg<sup>-1</sup> dry sediment. 20 different polymer types were found in total, and 97% of the total particles were smaller than 100 µm. The study demonstrates the capability to isolate, identify and quantify microplastic particles from sediments, and to differentiate particles into size classes between 10-500 µm. Particles > 500 µm did not give a representative picture of the particle composition. Polyurethane resins (acrylates) dominated small particles and polyamide fibers were most frequent among the larger particles. This study is the first to report concentrations of identified plastic particles from a Norwegian fjord, down to sizes below the limit of visual identification. The results provides a baseline for comparison for future investigations. Acknowledgement: The authors thank RFFVest for funding through pre-project RFFV#258890, project owner Bergen Kommune, and participating colleagues.

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Keywords: Sediment, polymer, quantification, i, FTIR, Distribution



## The role of bioturbation in distributing secondary microplastics in soft marine sediments

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Recent studies around the world show that high concentrations of microplastics are accumulating to seafloors. When deposited to the sediment surface, these particles may eventually get suspended back to the water column, buried under constantly settling new material, or transported deeper within the sediment column by bioturbation caused by benthic animal activities, such as feeding and burrowing. We studied the role of bioturbation in redistributing secondary microplastics from the sediment surface to deeper layers of sediment, and vice versa, from different sediment layers towards the sediment surface. These two mesocosm experiments were conducted with environmentally relevant concentrations of secondary microplastic fragments, which represented different materials (nylon and acrylonitrile butadiene styrene) and sizes (from 100  $\mu\text{m}$  to > 500  $\mu\text{m}$ ). Common benthic macroinvertebrates in the northern Baltic Sea (clam *Limecola balthica*, polychaete *Marenzelleria* spp., amphipod *Monoporeia affinis*) were used as bioturbators. Our results show that bioturbation plays an important role in transporting microplastics deeper in the sediment column, but does not markedly promote the return of buried microplastics to the sediment surface. Hence, the net transport of microplastics in the northern Baltic Sea sediments appears to be downwards, further supporting the idea of seafloors being the final sinks for microplastics. *L. balthica* was the only species ingesting microplastics in both studies, and the probability of ingestion seemed to decrease with the increasing sediment depth indicating that the burial of microplastics can lower the exposure to species feeding on the sediment surface.

Keywords: seafloor, secondary microplastic, bioturbation, Baltic Sea, invertebrate, ingestion

\*Speaker

## Assessing the overlap between plastics in seafloor habitats and ingested in species

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Microplastic ingestion has already been identified in fish and shark species around the

Mediterranean Sea (Fossi et al., 2018). More specifically, demersal species such as *Galeus melastomus* and *Mullus surmuletus* have been recorded to ingest microplastics with an occurrence varying between 17% and 27% (Alomar et al., 2017a,b). At a regional and local scale, some marine species have been proposed as bioindicators of marine litter contamination (Fossi et al., 2018). To study spatial trends of seafloor plastic as well as its impact on marine biota, integrated data from both ingestion occurrence in species and presence of plastic in habitats should be considered. In this sense a spatial overlap between seafloor plastic and plastic ingestion in marine organisms has been calculated for different depths around the Balearic Islands. A total of 44 experimental scientific bottom trawl hauls were carried out during spring. For each haul, plastic was quantified and a total of 54 species corresponding to the same bottom trawl hauls were analyzed for plastic ingestion. Natural factors such as submarine geomorphology, geographical settings and bathymetric stratification varied according to study areas as well as abiotic factors (fishing intensity, maritime traffic). Preliminary results showed that plastic abundance was higher in the west of the Balearic Islands and that areas exposed to higher anthropogenic activities, such as fishing, do reflect a higher overlap between seafloor plastics and ingestion occurrence in species (Figure 1). In addition, depths between 200 and 500 m had highest quantities of plastics which might have important ecological implications for key species living at these depths. Results from this study allow detecting species and habitats (mud, sand, maërl, rhodophytes, crinoids) more exposed and vulnerable to marine litter pollution.

Keywords: macroplastics, seafloor, species, ingestion

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## Current data does not confirm that deep sea sediments are the final sink for all microplastics. A meta-analysis.

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Plastics pollute marine environments at a global scale and occur in a wide range of sizes, mainly in the form of microplastics. In the ocean, abiotic degradation is limited and biodegradation is not likely to occur at significant rates, leading to the accumulation of this persistent pollutant. Despite growing plastic production and discharge into the environment, researchers have struggled to detect the predicted increases of marine plastic debris in sea surfaces, which has sparked discussions about

”missing plastics” and final sinks. The sinking of microplastics and eventual deposition into subtidal and deep-sea sediments has been hypothesized as a potential mechanism for plastic removal from sea surfaces. Here we show that this hypothesis was not confirmed after comparing the relative abundance of common polymer types from published surveys of different sampling locations. We found that, while intertidal and shallow subtidal areas (median depth 17.25 m) harbored similar proportions of synthetic polymer types, open oceans showed a clear segregation of plastics through the water column (i.e. surface, water column and deep-sea sediments). Polyethylene and polypropylene, both buoyant polymers (i.e. less dense than seawater) and among the most commonly manufactured plastic materials, dominated sea surface samples but decreased in abundance in the water column and were almost absent in deep-sea sediments, suggesting that deep ocean sediments are not the sink for these polymers. Despite the necessity to harmonize sampling and analytical methods, this meta-analysis demonstrates that microplastics segregate between sampling areas depending on the density of the polymeric material, and further research is required in order to determine the ultimate fate of two of the most abundant and recalcitrant synthetic polymers that pollute our oceans.

Keywords: microplastic, sinks, fate, distribution

\*Speaker

## Do microplastics in marine sediments affect inbenthic organisms?

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There is a huge discrepancy between the amount of plastics known to be introduced to the oceans and what can be accounted for in the water column. It is generally thought that most of the lacking plastics end up in marine sediments. It is therefore critical to clarify whether and how sediment-dwelling organisms are affected by plastics in sediment. Such assessment need to take into account modes of feeding and the life history of inbenthic organisms. This presentation will review existing knowledge including recent studies with a subsurface deposit-feeding bivalve, *Ennucula tenuis*, and a surface deposit feeder, *Abra nitida*. The available data suggest that particle densities currently found in coastal areas may affect the health of inbenthic organisms.

Keywords: marine sediment, microplastics, energy allocation, feeding mode. \*Speaker

# Tracing microplastics in aquatic environments based on sediment analogies

Kristina Enders \*† 1, Andrea Kappler 2, Oliver Biniash 3, Nicole Stollberg 1, Dieter Fischer 2, Klaus-Jochen Eichhorn 2, Falk Pollehne 1, Sonja Oberbeckmann 1, Matthias Labrenz 1

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Current projections of microplastics (MP) dispersal and deposition are subject to large inaccuracies. The mismatch between predicted and recovered MP numbers and with that the plastic's physical fate in the aquatic environment, has not yet been solved. We demonstrate the existence of analogies in distribution pattern between specific MP types and natural sediment composition, both being similarly influenced by hydrodynamic forcing. This study quantified and spectroscopically identified MP ( $\geq 500$   $\mu\text{m}$ ) in sediments of the Warnow estuary (southern Baltic Sea) and correlated MP distribution to hydrodynamic parameters and the effect of local sources. Significant correlations between specific sediment grain size classes and MP fractions were found, implying that MP distribution patterns can be largely deduced from simple sedimentological data, building upon the large knowledge reservoir of sediment transport mechanisms. Median grain size was the most accurate proxy obtained for high-density MP ( $r=-0.9$ ,  $p < 0.001$ ). Low-density particulate polymers showed a more complex distribution behaviour and require further research. The MP-sediment relationship in transport deposition behaviour exhibited an offset in size by averagely one order of magnitude, equating density differences with size. Based on sediment trap efficiencies the studied estuary potentially constitutes a permanent geographic sink to a substantial fraction of MP. Comparing complementary data from other studies revealed an influence of spatio-temporal connectivity of the system studied on the measure of fit. Spatial occurrences of type-specific MP were related to their corresponding place of emittance and served as a local source indicator; a waste water treatment plant and harbours, were identified. The importance of granulometric normalisation to eliminate hydrodynamic variabilities is highlighted, so that anthropogenic influences, over space and time can be determined. Thus, a close look at sediment distribution maps is of importance prior to sampling and during data interpretation when assessing an area's MP pollution level.

Keywords: sediment grain size, proxy analysis, normalisation, microplastic trap efficiency, source backtracking, FTIR & Raman microspectroscopy, plastic cycle, anthropocene

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## **November 22nd – Panel 16.2: 10h30' - 12h30' (AGH), Panel chaired by TBD**

### **A holistic approach to address plastic pollution on a local scale - The Roskilde Fjord case**

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Plastic pollution largely stems from peoples everyday use of plastic. This implies that even though the pollution has a magnitude where it can be characterized as a global problem, the main sources are found locally. Local solution can therefore play a dominant role in our efforts to change the current consumption patterns towards more sustainable use of plastic and thus reduce the environmental impact of plastic pollution. In the 3-year project presented here, we addressed local plastic pollution with a holistic and interdisciplinary approach in semi-closed estuarine water body – Roskilde Fjord, Denmark. The aim of the project was to characterize the magnitude, composition and impact of plastic pollution in the fjord along with dominant exposure routes to the water body. The project furthermore worked with local stakeholders, including citizens, authorities and wastewater treatment plants with the aim of identifying suitable solutions to the plastic pollution. The interdisciplinary approach, drawing upon science, social science and humanities generated a comprehensive understanding of pollution patterns and potential impacts and furthermore initiated different mitigation processes such as citizen science activities, political attention and enhanced focus on point sources.

*Keywords: Plastic pollution, citizen science, ecological impact, point sources, vector effects*

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## **University Freshmen's Conceptions about Microplastics in Aquatic Ecosystems**

Tabea Loermann 1, Franz Bogner \* 1

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Individual conceptions about a selected topic may originate in classroom learning or daily

experiences, such as interactions with news and media. Consequently, individual ideas may not follow scientific correctness or even may be flawed. The latter situation are also referred to as alternative or intuitive conceptions (Calik & Ayas 2005). The knowledge concerning alternative conceptions of any teaching purpose is relevant in order to specifically promote the intended aspects of lessons or modules (e.g. Franke & Bogner 2011; Franke & Bogner 2013; Fremerey, Liefländer, Bogner 2014; Schmid & Bogner, 2018). Currently, the topic of microplastics is a frequent issue in the general public. Almost on a daily basis new contributions feed discussions. Thus, a solid knowledge about conceptions would help to clarify fine-tune efforts for module plans and that is why we applied a first survey about conceptions. Respondents were freshmen who successfully has passed secondary schools (age 19-20 years). They were asked to explain the term microplastic, to describe its origin and to label potential sources in households as well as to indicate its occurrence in aquatic ecosystems. Finally, the respondents should assess the potential danger of microplastics. This study is part of the three-year BMBF-project PLAWES which monitors microplastic contamination in a broad scientific approach at the model region Weser - Wattenmeer National Park (Germany). One specific work package concentrates on the issue of communication: a) to record and categorize conceptions of pupils and adolescents; b) to incorporate the extracted conception architecture into teaching efforts as well as to develop exemplarily appropriate education modules. Finally, c) to feed a teaching-learning internet portal mainly for science teacher but also make it available to the general public. Our presentation demonstrate first results mainly to a) and b) which currently are in depth analyzed.

*Keywords: conception, adolescents, scientifically correct. intuitive conceptions*

*\*Speaker*

## Principe Island UNESCO Biosphere Reserve - Towards a plastic free Island in 2020

Miguel Clüsener-Godt 1, Antonio D. Abreu \*1

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Following the launching in 2014 of the campaign: "No Plastic, a Small Gesture is in Our Hands", around 1 million plastic bottles were collected in Príncipe Island UNESCO Biosphere Reserve (Democratic Republic of São Tomé & Príncipe, Gulf of Guinea) and exchanged by reusable Biosphere Bottles. Awareness and capacitation in schools and communities lead to a significant engagement and impact with the delivery of 7000 Biosphere bottles, within a population accounting to 8.000 inhabitants, during the 3 years of the Water & Recycle Project. The collected

plastic was compacted and exported for recycling and/or valorisation in Portugal. The Water & Recycle Change is an initiative promoted by Príncipe Island UNESCO Biosphere Reserve as the result of a partnership between the Regional Government of Príncipe Autonomous Region, UNESCO's MAN and Biosphere Programme, The Spanish Ministry of Agriculture, Food and Environment and the HBD Group.

Within the Scientific Expedition BioPríncipe 2016, aiming to assess and mapping marine and coastal habitats and biodiversity of Príncipe Island, microplastic surveys were conducted on the beaches of Príncipe Island, providing a first assessment of its contents in sediments.

In 2016 the project evolved towards a more comprehensive integration of waste management, leading to the introduction of glass recycling and compost production.

Next steps will include legislation that will be produced by the Regional Government and Parliament with arrangements to eradicate the introduction of one-time use plastic in Príncipe Island leading to the major goal to bring Príncipe as a "plastic free" island by 2020.

*Keywords: biosphere reserves, Principe Island, plastic free island, UNESCO*

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## "OceanWise - Promoting social change"

Flávia Silva \* 1, Lia Vasconcelos 2, José Carlos Ferreira 2, Ricardo Resende 2

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Expanded polystyrene (EPS) products entail great risks for marine environment, and the common presence on EU Atlantic coasts is an issue that needs to be addressed. Large amounts of objects derived from this material can be found on beaches, as small plastic/polystyrene pieces constitute 30% of total items, according to OSPAR beach monitoring surveys. EPS composition greatly contributes for the problem at hand since its weight/volume ratio is very small and the product itself is non-biodegradable, thus it easily fragments into microplastics that persist in the marine environment. Considering these, OceanWise project aims to develop a set of long-term measures to address the impacts of EPS products in the North-East Atlantic Ocean. It is intended to generate new and best practices within sectors using, manufacturing or recycling EPS, supported by resource-efficiency, participatory methods and Circular Economy principles. To prosecute these objectives, a consortium composed of 13 partners from 5 European countries (Portugal, Spain, Ireland, France and United Kingdom) was created, uniting national governmental agencies re-

sponsible for marine environment, waste management and recycling companies, and academics.

Therefore, the challenge implies identifying EPS products more likely to reach the marine environment, explore plausible options to achieve better environmental outcomes within different sectors, engage producer and designer communities on the sustainability of specific applications exploring more circular models, and develop CE-oriented methodologies to assess new opportunities, barriers and policy options. A strong component of this project is based on the mobilization of stakeholders. OceanWise will explore methodologies of emancipatory participation, enhancing interaction and collaboration between people from various sectors that hold an interest on the project's objectives. By involving and engaging stakeholders from various backgrounds of the society, it is expected to not only raise awareness but also promote social change towards the use of EPS products.

Keywords: EPS, marine environment, stakeholders engagement, North, East Atlantic, circular economy

\*Speaker

## “Less Plastic more Mediterranean”: a Research and Awareness campaign in the Mediterranean Sea.

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1 National Research Council – Institute of Marine Sciences (CNR-ISMAR) – Italy; 2 Greenpeace, Rome, Italy – Italy 3 Università Politecnica delle Marche (UNIVPM) – Italy

In Summer 2017 the international tour “LESS PLASTIC MORE MEDITERRANEAN” has been carried out by GREENPEACE to gather direct data on plastic pollution and to inform public opinion about this environmental emerging issue. During the Italian part of the tour an extended microplastics (MPs) investigation was carried out: water samples were collected using both Manta and Plankton nets (mesh size 330 and 200  $\mu\text{m}$ ) from 17 sites along the Italian coasts, characterized by different level of anthropogenic pressure (from ports, river mouths to MPAs). Moreover, depending on the availability, marine organisms were also collected in collaboration with local fishermen. Water analyses confirm MPs presence in all the stations with an average of 0,52 items/ $\text{m}^3$ . The maximum value (3,56 items/ $\text{m}^3$ ) was observed in Portici (a highly stressed site) but high MPs (2,2 items/ $\text{m}^3$ ) was even found in the Tremiti Islands MPA suggesting that these isolated systems are subjected to water flow dynamics able to enrich local biodiversity but even to make



pristine areas potential hot spot for MPs accumulation.

Organisms investigation pointed out that MPs ingestion is a widespread phenomenon in the Tyrrhenian Sea with 25-30% of positive organisms on 201 analyzed; slight geographical differences occurred in terms of size and typology of ingested particles (mainly PE, EVA and PP) and a no clear relationships was observed between microplastic ingestion in different species and trophic position, feeding strategy or habitat preference. A special focus is given to the results obtained on the Giglio Island, where a significant recovery of microplastic pollution has been observed in fish (95% vs 35% of fish positive to the MP ingestion), three years after the Costa Concordia wreck removal. This study underlining the importance of collaboration between the Research Institutions (involved in European project on microplastics, i.e. JPI-Oceans) and the non-governmental organization such as GREENPEACE.

Keywords: Mediterranean Sea, Giglio Island, microplastics, water, fish.

\*Speaker

## A social-ecological framework tackling plastic pollution

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Since plastic's invention for mass use in the 20th century, scientists and decision-makers have now come to recognize plastic pollution as a global problem. Due to the complexity of the issue, a holistic perspective is required to understand plastic contamination processes and find solutions. To participate in the common effort towards such a perspective, we have developed a research protocol using the social-ecological system concept (Berkes and Folke, 1998; Ostrom, 2009) following the procedure developed by Schlüter et al. (2014). The procedure consists of (i) identifying a set of relevant indicators, (ii) studying them to understand the processes involved in causes and consequences of plastic pollution, (iii) identifying variables, concepts and relationships involved in those processes in order to (iv) design a conceptual model and, in the end, to (v) build a formal dynamic model to test plastic management scenarios in terms of potential ecological, social and economic impacts on social-ecological systems. Our aim is to understand how the choice of plastic management scenario influences social-ecological systems. In the dynamic model, we consider delays between socio-economic causes and ecological consequences and their feedback impacts. The reciprocal effects of social and economic inequities on plastic management measures are also

considered. We give particular attention to plastic management measures that enable decoupling between plastic pollution and socio-economic development, a necessary condition to achieve sustainable social-ecological systems, including polycentric governance of plastic management (Ostrom, 2010; Benkler, 2011).

Keywords: Social, ecological systems, Plastic, Pollution, Dynamic model, Governance

\*Corresponding author: ueharatakuro@gmail.com †Speaker

## Plastic in the Environment – Sources, Sinks, Solutions

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The global challenge of tackling plastic litter has received increased international attention over the last years. In particular the pressing issue of marine litter has been highlighted prominently in action plans of both G7 (2015) and G20 (2017) as well as 2015 in the United Nations Sustainable Development Goals (SDGs) in SDG 12.5 and 14.1. However, knowledge gaps on the environmental impact of (micro-)plastics on aquatic ecosystems persist that impede implementing these agreed-upon objectives. Taking this into account, the German Federal Ministry of Education and Research (BMBF) set up the research focus “Plastic in the Environment” in 2017. The research focus aims at providing an overall picture of the environmental impact of plastics along the entire plastics lifecycle from manufacturing to consumption and to disposal. In doing so, it seeks to assess scientifically the problem of plastic waste in its entirety and to fill existing knowledge gaps. In addition, concrete solutions to reduce the input of (micro-)plastics into the environment will be identified and implemented. More than 100 institutions from science, industry and practice are involved in what is currently the world’s largest research focus in the field of environmental impacts of plastics. Between 2017 and 2021, a total of 18 joint projects and a scientific accompanying project will be funded with around 35 million. The research focus covers five diverse issue areas along the entire plastic lifecycle: green economy, consumption, recycling, freshwater ecosystems and saltwater ecosystems. This holistic approach allows for a better understanding of the environmental impacts of plastic waste from river basins to oceans. With 8 out of 18 projects being in the area of freshwater ecosystems, the research focus puts special emphasis on increasing knowledge on entry paths, dynamics and effects of microplastics in these ecosystems as well as on possible ways of reducing them.

Keywords: Plastics, Microplastics, Freshwater, Marine, Plastic lifecycle

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# Putting the ‘Work’ back into Workshop – the EPHEMARE stakeholder workshop on microplastics in the marine environment

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The JPI Oceans-funded EPHEMARE (Ecotoxicological Effects of Microplastics in Marine Ecosystems) project invited 30 representatives from the fields of Industry, Research/Science and Regulation/Policy to Antwerp, Belgium, on February 21st, 2018. The workshop showcased the latest research findings on the topic, within the context of the existing and emerging EU regulatory, policy, and legal landscape; identified future research questions considering all stakeholders’ needs; and facilitated collaborative work to identify potential solutions - addressing adverse impacts of microplastics on marine life - that are deemed effective and implementable. Workshop participants learned about opportunities to provide scientific input on microplastics to the new cycle of monitoring for the MSFD Task Group on Marine Litter, towards the implementation of the newly-released Plastic Strategy, and the ECHA communication concerning REACH restrictions for microplastics intentionally added in products. EPHEMARE scientists highlighted that detection of micro- and nanoplastic effects may require dedicated approaches which are beyond the current standard methods (e.g. effects on animal behaviour), and the need for Environmental Quality Standards (EQS) for different size classes and types of plastics. The future direction of microplastic research was discussed in break-out groups of five, and was reported back to the room. Finally, groups worked together to formulate potential solutions to address adverse impacts of microplastics on marine ecosystems. The workshop adapted the World Café method (<http://www.theworldcafe.com/>), to create an environment fostering collaboration and inclusivity. Workshop participants voted as individuals and anonymously on the in the group work, identified potential solutions under the categories of perceived (a) effectiveness and (b) feasibility, to allow expression of individual opinions. Outcomes of this workshop, such as the current state and the future direction of microplastic research, were summarised in a briefing note, which has been utilised to inform funding agencies and policymakers.

Keywords: stakeholder engagement, project workshop, science policy interface, science industry communication, future research direction for micro, plastics

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## **November 22nd – Panel 17.1: 14h30 - 16h (AGH), Panel chaired by Julie Forgues, Bettina Hutschek and Lionel Jaffrès.**

### **Communicating Plastic Pollution through Art**

Swaantje Guentzel \* 1

1 Swaantje Güntzel / artist – Barnerstrasse 35 22765 Hamburg, Germany

For almost 10 years now the Hamburg based German artist Swaantje Güntzel (\*1972) has been dealing with the various facets of anthropogenic pollution of the oceans with an emphasis on phenomena such as the Great Pacific Garbage Patch, the emergence of the plastisphere and microplastics. She works conceptually across a range of different disciplines, much of her work is inspired by scientific research based on ongoing collaborations with marine biologists all over the world who provide Güntzel with data and material. One of the main focuses of her work is to track down the origins of marine pollution and to bring them into public imagination. Her work aims to give an impulse to the audience to question the state of their surrounding environment, to analyze our polluting lifestyles overwhelming the natural world, its consequences for marine life resulting therefrom and our inability to address this global problem. Through the example of her work she will illustrate the possibilities but also the limits to raise awareness on plastic pollution. She will talk about her experiences dealing with the different audiences that are "confronted" with her artworks going from gallery visitors to unwitting participants of performances presented in public space and the respective feedback.

Keywords: art, environmental art, awareness, microplastic, plastic pollution

\*Speaker

### **Simple Visuals = Powerful Messages = Real Change**

Muntasir Mamun \* 1

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Debris is not local anymore and most of our watersheds, waterways, rivers and oceans are getting land based pollutants by our habitual activity. Debris removal programs are often lead by government organizations or nongovernment organization but in my study if integration is possible among govt, corporate and local people, managing beaches and marine debris are way easier,

effective and sustainable. Additionally, the quantity and quality of data can be more authentic and can reduce removal efforts by implementing Smart Phones. This technology can identify or locate exact location of debris by beachgoers, dedicated volunteers or any other parties alike. Special application (free to use and easily downloadable from multiple sites) developed for Android based Smart Phones to identify fixed and floating marine debris visible on the beaches and can be shown on Google Earth/Map. It helps cut down the cost of collection of debris.

A simple visual of the trash can gradually unfold many different ways of our contribution to micro debris. The human behavior of littering trash can identify a sudden or fundamental transformation of economic, social, geopolitical and almost every aspect of our society. The individual's habit, behavior, experience and knowledge on the impact of their contribution can be addressed by a simple method, visualization. It can generate and convey a powerful message in a most simplified form. Sometimes, we don't need a lot of complex calculations to get a point across. Honest, direct message can convince critical mass to address the problem since they are the one who contributes the most. This project involves discovering the human behavior on littering across USA, from west to east coast. A 5000km long bicycle ride to aware how the waste that we generate here can have a profound impact in places very far away.

Keywords: : Bangladesh, Cox's Bazar, Android Smart Phones, Application, Google Map, citizen science, flock sourcing  
\*Speaker

## Marine Plastic Pollution in the Media: A Survey on German Daily Newspapers 2007 – 2017

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Marine plastic pollution has been a matter of scientific investigation for over four decades. In the media, however, the attention to this subject raised only in the last few years. We analyzed the quantity and quality of reports on marine plastic pollution in six German newspapers from 2007 to 2017 including the national "Süddeutsche Zeitung" and "taz" ("Tageszeitung"); two local newspapers ("Hamburger Abendblatt", "Nürnberger Nachrichten"), one tabloid ("Express") and one professional journal ("VDI-Nachrichten of the Organization of German Engineers"). In total, 236 articles on the subject were detected within the evaluation period. Their number rose continuously from as few as two articles in 2007 toward the maximum of 55 articles in 2017. The major increase numbers appeared in 2013. The major topics were 1) projects/ideas to remove plastic from the sea, 2) general information on marine plastic pollution, 3) recycling and substitutes for plastic, 4)

microplastics, 5) Plastic bags. Preferred journalistic genres were background stories (37%) followed by reportages (24%) and news reports (23%). In general, all articles pointed out problems and potential hazards associated with marine plastic pollution. However, until 2016 the numbers of articles tending to be optimistic vs those being pessimistic was more or less balanced. "Optimistic" is defined as e.g. reports on research, projects, or initiatives aiming at practical or political solutions. "Pessimistic" means describing the problem as hardly solvable. In 2017 twice as many articles had a pessimistic view than an optimistic one. Continuous increase in coverage is, supposedly, due to constantly increasing public awareness. The peak as well as the "change of tone" in 2017 might be due to major events like the G7- summit of that year and the first ever UN Ocean Conference. Both addressed marine plastic pollution but, according to some environmental groups, did not bring about enough tangible measures.

Keywords: Marine Plastic Pollution, Media, Press Reports, coverage timeline

\*Speaker

## Telling stories about (micro)plastic pollution: Media images, public perceptions and social change

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Microplastic pollution is now in the public domain as an emerging issue of global concern however there is little known about how this issue is framed within media reporting and also a dearth of studies exploring public understandings of the issue. In this paper, we explore how ideas about microplastics are mediated by culturally embedded notions of what is 'risky' or 'healthy'. Specifically, we explore the wider role of media in telling stories and creating "frames of understanding" about the environmental and health risks of (micro) plastics. This paper builds on a unique interdisciplinary study at Brunel University London. The project involved eco-toxicologists, sociologists, environmental scientists and natural history film makers and used the adventure documentary film about the problem of single-use plastics, 'A Plastic Ocean', Netflix, 2017 to explore wider ideas about the problem. This project is the first to systematically analyse media content (across 1 year, 2014-15) and we also used online deliberative survey tools involving a nationally representative sample of the UK population to explore how people engage with media stories about the topic. Additional rich qualitative data were generated in 6 focus group sessions (involving water sports club members, arts students, community workers and young mothers living on a budget). The paper concludes by highlighting how wider public perceptions of risk and health

intersect with and may potentially also undermine messages regarding possible solutions to this problem and how we must learn from previous studies of media, science and publics if we are to develop culturally appropriate strategies to shape behaviour.

Keywords: media, trust, social change, public, science communication, popular media

\*Speaker

## Marine Debris: art, science and education to awaken environmental values

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A transdisciplinary dialogue between artists and researchers of the Rio Grande Federal University in Brazil allowed the unification of art and science to provoke sensibilization towards a pressing environmental issue. A large itinerant structure was collectively built to artistically exhibit the marine debris problem, with a special focus on the "Plastisphere". This structure consisted of a bamboo geodesic covered with litter collected at a local beach, and inside the geodesic, visitors were immersed in the universe of the Plastisphere, through the presentation of hanging images of epiplastic organisms from microplastic photographed with Scanning Electron Microscopy (magnification up to 18,000x). The geodesic symbolizes a new consciousness that unites ecology and sustainability; through it, we bring focus to the excessive waste produced by modern consumption, that floods the oceans with trash. Surrounded by this waste that we generate, we question our choices as a society. Images invite us to transit the micro and macro- scopic, and perceive the grave consequences of our neglect, from the smallest intangible particle to the intolerable weight of litter, so useful as objects of desire and so useless when disposed of. Visitors are reminded that all this waste carries life, and memory of life. The geodesic structure was shown at a shopping mall, creating a contrasting environment within aseptic halls. It was presented in Portuguese and sign language, with information written in English, Spanish and French, and received hundreds of visitors. This is the first artistic-scientific initiative on the Plastisphere to ever be exhibited in Brazil; the integration of arts and science should be further explored to increase awareness on marine debris.

Keywords: Marine litter, Environmental education

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# Consumer responses to single-use packaging

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**Introduction.** Plastic litter in the ocean is causing harm to the marine wildlife and ecosystems. Single-use food and beverage packaging items make up a large share of the marine plastic pollution. These items are also a source of secondary microplastics as they break down into smaller fragments over time. Packaging items accumulate in the ocean as a result of inappropriate disposal and spillage from waste landfills. One source of the problem are unsustainable consumer decisions, as food and beverage items packed in plastic that is often unrecyclable are being purchased daily. In order to effect change in consumption behaviour it is important to understand what motivates purchasing decisions. This study explored the perceptions and attitudes underlying consumer responses to packaging. Of particular interest was to examine whether concern and passion for the ocean are associated with preferences for ecological packaging.

**Method.** 60 students took part in a computer experiment in which their responses (willingness to buy and affective reactions) to different beverage products were recorded. 24 pictures of beverages with unique combinations of packaging recyclability (recyclable or non-recyclable), material (plastic, glass, aluminium or carton) and content (water, orange juice or cola) were shown to all participants. A survey measuring marine litter concern and ocean connectedness was also administered. The data were analysed using linear mixed-effects models.

**Results and Discussion:** Recyclable packaging was rated higher for willingness to buy and positive affective reaction than non-recyclable packaging. Ratings for plastic and glass were higher than those for aluminium or carton, but there was no difference between plastic and glass. Those with higher marine litter concern and ocean connectedness differentiated more between recyclable and non-recyclable packaging (Figure 1), whereas no similar interaction effects were found for material. Thus, our passion for the ocean could potentially be harnessed to target and change consumption behaviours.

\*Speaker

Keywords: packaging, plastic, single, use, recyclable, packaging material



# Microplastics and Medusae – Expeditions into H<sub>2</sub>O. Art as a “mastic medium” to foster the interdisciplinary exchange on plastic pollution of aquatic environments.

Roman Kroke<sup>1</sup>

*Interdisciplinary Artist, lecturer at the Berlin University of the Arts (UdK)*

In comparison to other means of mediation, what intrinsic value may be attributed to the arts as a bridge of communication conveying scientific research to a broader public, for instance with respect to fostering ecological awareness and impacting daily consumer habits? From a scientific viewpoint, could the examination of artistic practice provide new impulses for thought processes and serve as a catalyst for an experimental exploration of new methodologies? In reverse, what discoveries could artists make with respect to their creative language by immersing themselves in the world of micro-science?

Since 2016, Roman Kroke has been exploring these questions starting with several research travels through France and Germany exchanging with scientists currently invested in the topic of microplastics. He also met up with contemporary philosophers working on the element "water", the materiality of "plastic" as well as the concepts of "sustainable development" and "responsible citizenship". Topics of particular interest: the relationships between "fragility and responsibility", "the visible and the invisible", "interiority and exteriority", "man and matter" as well as the "evolving polarity between the natural and the artificial".

In the lecture, Roman Kroke will provide an evaluative overview of his interdisciplinary projects realized on the basis of this research: (1.) A series of drawings in which scientific, philosophical and literary sources enter into a metaphorical dialogue about the topic of microplastics; (2.) An artistic-scientific “hybrid-seminar” directed for a mixed group of students of the Berlin University of the Arts and the Technical University Berlin – in partnership with 13 German laboratories specialized in the research on microplastics ; the artworks created in this seminar will have their exhibition première as part of the festival “The Universal Sea – Pure or Plastic?!” in Budapest (October 2018); (3.) A workshop for high-school students realized as part of the international scientific colloquium EcoBIM 2018 (University of Bordeaux, France).

## **November 22nd – Panel 17.2: 14h30 - 16h (AGH), Panel chaired by Arnaud Huvet.**

### **Microplastics: an unexplored journey inside zebrafish embryos**

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Problems related to microplastics (MPs) and their toxic effects on aquatic organisms are currently in the eye of the storm. Among all discussions, a crucial issue still remains mostly unresolved: once being ingested, where do MPs go? This preliminary study aims to investigate the MPs' journey inside zebrafish (*Danio rerio*) embryos exposed at 72 hours post-fertilization (hpf) until 120 hpf to 50 mg/L of virgin polystyrene microbeads with a diameter of 0.5  $\mu$ m. First of all, we selected the advanced microscopy technique more suitable for our purposes among light sheet microscopy, two-photon excitation microscopy and confocal microscopy (Figure 1). Finally, we decided to use confocal microscopy on cryostat sections to evaluate not only the ingestion of MPs, but mainly their possible tissues infiltration. Moreover, a suite of biomarkers was applied to evaluate toxic effects of MPs on the embryos. The oxidative stress was assessed by the analysis of protein carbonyl content (PCC) and the activity of antioxidant/detoxifying enzymes (GST, CAT, GPx and SOD), while the activation of the detoxification system was assessed through the analysis of P-gp transporter efflux.

Our data clearly proved the MPs' uptake, distinctly showing beads inside the gastrointestinal tract, with some of them apparently migrating to other tissues, with potential cellular and molecular consequences. Biomarkers analysis revealed effects of MPs, suggesting the activation of zebrafish embryos defence system. Although still preliminary, our results highlight the need of more deepened investigation on MPs infiltration and consequent potential toxicological effects, for instance with longer exposure times and measuring different endpoints. This study represents the first step of a wider project, that will be also aimed to the evaluation of MPs' potential Trojan horse effect when in presence of other environmental pollutants.

Keywords: Microplastics, zebrafish, advanced microscopy, toxicity, biomarkers

\*Speaker

## Differential effects of pollutant-spiked microplastics on two model fish, zebrafish and marine medaka

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The growing production of plastics increased the amount of debris in aquatic ecosystems. Their degradation leads to the emission of microplastics (MPs) in the environment. Virgin plastic polymers (industrial plastic) are, in principle, biologically inert and thus non-toxic. However, plastic production includes additives (plasticizers, colorants or fire retardants) that can be toxic. In addition to the potential toxicity caused by plastic additives, MPs offer a surface where many pollutants can adsorb, including persistent organic pollutants. In the marine environment, such chemicals can be found at the surface layer, where low-density microplastics are most abundant as well. Accumulation of MPs in aquatic ecosystems became an emerging problematic, but the toxicity of MPs for wildlife is poorly understood. Therefore, objectives of the present study were to investigate the possible toxicity of virgin MPs and the same MPs spiked with environmental concentrations of organic pollutants (PFOS, BaP or oxybenzone). Embryo-larval stages (non-protected stages according to regulation) of two teleost fish models were exposed to virgin and spiked MPs to evaluate the influence of exposure duration and water salinity:

- zebrafish (*Danio rerio*): freshwater and short development (96 hpf, at 26°C)
- marine medaka (*Oryzias melastigma*): marine water and long development (13 dpf, at 26°C).

Acute toxicity (survival, hatching rate), biometry, genotoxicity, in vivo EROD activity, and locomotor activity (photo-motor response, PMR) were investigated to characterize the potential toxicity of MPs.

No toxicity was observed using zebrafish embryos and larvae exposed to virgin or spiked MPs, but sub-lethal effects such as delayed hatching or hypoactivity were observed using marine medaka exposed to spiked MPs. In parallel, the same experiment using environmental MPs from Guadalupe

was conducted on zebrafish embryos and larvae, but no toxicity was found. These results suggest that a longer exposure is likely required to assess toxicity of MPs and associated pollutants.

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Keywords: Zebrafish, marine medaka, early life stage exposure, embryotoxicity, microplastics (MPs), organic pollutants

## Contamination of the ichthyofauna by the microplastics in the catches of some species in the southern Cameroon coast

David Mboglen \* 1

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Aquatic environments are increasingly subject to pollution, especially plastics. The pollution of the marine environment by plastic materials is increasing; the quantities produced today are 170 times higher than it was 60 years ago. It is estimated that nearly 10% of this plastic is poured into the oceans where it accumulates without being degraded. Fragmentation of plastic debris leads to the formation of microplastics which are likely to be ingested by aquatic fauna. The objective of this study is to determine the presence and abundance of plastic debris in the catches of *Pseudolithus senegalensis*, *Pseudolithus typus* and *Ethmalosa fimbriata* in the dockyard of Londji and Mboa-manga in the Southern Cameroon region. In the context of increased plastic production, the implications will be that of the presence of microplastics on the feed of marine fish which will definitely corresponds to a modification of the diet composition and the trophic ethology of *Pseudolithus senegalensis*, *Pseudolithus typus* and *Ethmalosa fimbriata*. It is in this context that, 372 fish were sampled at the dockyard of Londji and Mboa-manga respectively in Kribi I and Kribi II districts. The stomach contents of all samples were treated and examined according to the method described by Collard et al (2015) and then examined under a microscope. The results indicate that the trophic ethology of the fish sampled, particularly in adults who confuse them with their natural feed. From this conclusion, it's possible to think that all the ichthyofauna caught along the coast of Cameroon are prone to contamination by ingestion of microplastics. Further research should make it possible to identify the consequences of this pollution on marine organisms, but also on other pollutants present on plastics in general.

Keywords: Food composition, ichthyofauna, marine pollution, microplastics

\*Speaker

# Effects of small-sized plastics in marine organisms: mussels and fish

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The existence of small-sized plastic waste, such as microplastics and nanoplastics (NPs) in the marine environment and its potential impact on aquatic life has recently become a major concern due to the large quantity of plastic debris released in coastal areas. The present study aimed to evaluate the effects of NPs on two of the most commercially important species in the Mediterranean area, *Mytilus galloprovincialis* (Mediterranean mussel) and *Dicentrarchus labrax* (European seabass). As edible species, they may represent an important route of entry of small-sized plastics into humans. For that purpose two experiments were carried out and mussels or fish were exposed during 96 h to a concentration range of nanoplastics (polystyrene and poly-methylmethacrylate). Results showed that in mussels, the exposure to 0.5 mg/L NPs induced an increase in the total oxidative status in the digestive gland. Increased total antioxidant capacity and esterase activity were observed for 50 mg/L, in digestive glands and gills, respectively. NPs exposure also induced an inhibition of cholinesterase activity and genotoxicity in hemolymph. Moreover, lipid peroxidation was observed in the digestive gland for 0.05 mg/L exposure, showing that NPs can induce oxidative damage at low levels. The present study demonstrated that NPs, even at low concentrations, led to alterations on the assessed mussels' endpoints. Concerning results in fish, it was observed an alteration on molecular signaling pathways related to lipid metabolism, as well as alterations in biochemical endpoints, such as decreased esterase activity, suggesting that the fish's immune system might be compromised by exposure to NPs. Present results suggest that NPs may represent a hazard to marine species.

Keywords: Nanoplastics, marine environment, mussels, fish, gene expression

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## Physiological consequences of chronic exposure of marine medaka to microplastics

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Occurrence of microplastics (MPs) in fish digestive tract varies among species but can reach 75% of the individuals. Toxicity of MPs remain poorly understood/known and can be of physical or chemical origin. Chemical toxicity can be intrinsic (additives) or extrinsic (adsorbed pollutants), the importance of the later still being debated compared to other sources. We have performed long-term trophic exposures of marine medaka (*O. melastigma*) to MPs (polyethylene 11-13µm) either virgin or adsorbed with environmental concentrations of organic pollutants (PFOS, BaP or benzophenone-3 (BP3)). MPs were spiked in diet at 1% w/w. Exposure started at first feeding and lasted 4.5 months. Molecular (genotoxicity, oxidative stress, endocrine status) and individual (survival, growth and reproduction, behaviour) variables were monitored throughout exposure. We observed:

- No evidence of genotoxicity or oxidative stress
- A decrease in female growth of all MP conditions
- A delay in reproduction onset and a decrease in reproduction output of medaka exposed to MP-PFOS and MP-BP3
- In the case of MP-PFOS, this was associated to an increase in circulating estradiol concentration in both females and males.

These results suggest that life-long exposure to MPs can trigger physiological disruptions in fish which may impair recruitment and population sustainability.

\*Speaker

Keywords: organic pollutants, fish, physiology, reproduction, behaviour

# Investigating marine litter in remote Arctic Islands

Eelco Leemans \* 1

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From 31 May – 7 June 2017 Eelco Leemans and Wouter Jan Strietman investigated the litter washed up on remote beaches in the Arctic. Mostly pieces of plastic, fishing nets and rope were found there. Information about the origins of the litter is crucial for effective measures at the source. On Jan Mayen, the most remote island in the North-Atlantic Ocean, the total number of litter items on a 100 meter stretch of beach was 575, while this amount reached 876 items on Svalbard. In comparison, the average number of litter items on Dutch beaches is 375 per 100m beach.

An important part of the encountered beach litter is related to the various kinds of fisheries taking place around Jan Mayen, Svalbard and the Barents Sea. Another part is transported by currents from Europe or Siberia, or comes from melting pack ice. On some beaches there was still some ice or snow, so the total amount of litter may well be higher than the actual count.

The survey was based on international monitoring guidelines, whereby the amount and types of litter is analysed over a stretch of 100 m beach. With this analysis and through dedicating the sources of litter, stakeholders and governments can take measures to reduce the plastic pollution in the Arctic. Plastic litter in the Oceans has negative effects on the vulnerable Arctic ecosystem: animals become entangled in nets and ropes or eat plastic and ingest endocrine disruptors. Based on discussions with local stakeholders, there is a need for further research into the exact sources of litter on the beaches of Svalbard and Jan Mayen, in particular on unsurveyed beaches. The researchers see possibilities in cooperation with these parties, Norwegian researchers and the Dutch government to roll out a source-based approach.

Keywords: Arctic Svalbard Jan Mayen

\*Speaker

## **November 22nd – Panel 18.1: 16h30 - 18h30 (AGH), Panel chaired by Tania Montoto.**

### **LITTERBASE: An Online Portal for Marine Litter and Microplastics and Their Impacts for Marine Life**

Mine B. Tekman 1, Melanie Bergmann 1, Lars Gutow 1, Corina Peter \*† 1

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Plastic pollution of the oceans is a global problem, which currently receives increasing attention by policy makers, public authorities, media and the general public. Although this field has recently seen a marked increase in research efforts, there is a level of uncertainty, misinformation and worry in the general public indicating that scientific knowledge is currently insufficiently channelled back to society. To bridge this gap, we devised an online portal, LITTERBASE ([litterbase.org](http://litterbase.org)), which provides continuously updated information on the global distribution and composition of litter pollution and its impacts on biota to stakeholders based on data from peer-reviewed publications. To date, data from 1,725 studies (status April 2018) have been extracted, fed manually into a database and translated into understandable global maps and infographs to open scientific knowledge to the public. Bibliometric data of all publications were entered, as were metadata pertaining to litter type (e.g. plastic, glass, metal, fishing gear), size (i.e. nano, micro, macro), litter quantity unit (e.g. items km<sup>-2</sup>, items km<sup>-1</sup>, items m<sup>3</sup>), aquatic system (e.g. marine, freshwater, estuary), biome (e.g. beach, sea surface, water column, benthic) and total litter quantity. Litter quantities were standardised to the most frequently used units to achieve comparability. Data on biological interactions with litter were also extracted: location of field records, number of species affected, percentage of individuals affected, type of interaction incurred (e.g. entanglement, ingestion, coverage, rafting), effects on biota (e.g. injury, mortality, growth, behaviour), litter type, size, aquatic system and biome. Accordingly, 1,472 taxa (status April 2018) have been found to interact with marine litter. Here, we will discuss the use of LITTERBASE with a focus on microplastic distribution, particularly in terms of unveiling our blind spots.

Keywords: database, macrolitter, microplastics, effects, knowledge transfer

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# Lessons from the past: Using Machine Learning to mine the literature for past observations of marine litter

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The past few years have seen a rapid increase in the number of scientific publications reporting on the prevalence of plastic litter in the marine environment. For a full picture of the distribution of this plastic litter, and in order to do meta-analysis, it is important to combine these observations into one large dataset. There have been targeted programs to do literature searches on marine litter in specific parts of the system, such as the surface ocean or within the guts of certain taxa or regions. There are also attempts to combine all observations of plastic into one large database structure; the LITTERBASE at AWI being the most well-known of these. However, maintaining this database, and expanding it with newly published research is a significant task. Furthermore, it is near-unfeasible to go through the entire back-catalogue of scientific publications on the topic to find any observations of marine litter.

Here, we present a new project to use Machine Learning and Natural Language Processing to automatically mine the past (and newly published) literature for any observations of marine plastic. The algorithm is trained with the curated entries from LITTERBASE as gold standard. Meta-data such as location, units and method are automatically parsed and fed into a database. This database can then be used to study spatial patterns and temporal trends of marine litter in all forms, including on the sea surface and sea floor, on coastlines, and in marine organisms. The database is open source and accessible publicly for anyone to contribute to and use.

Keywords: marine plastic litter, observations, sampling methodology, global

\*Speaker

# Sustained ocean observations of marine plastic contaminants as an essential component of the Global Ocean Observing System

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The Global Ocean Observing System (GOOS) plays a critical role in the ever-evolving relationship between the ocean and society by coordinating a distributed observing system to serve global and local needs. GOOS delivers a systematic approach to ocean observing by identifying the essential information needs for a wide range of end users and ensuring delivery in a sustained and responsive way. While requirements for sustained ocean observations are well-defined for almost thirty Essential Ocean Variables (EOVs) spanning physics and climate, biogeochemistry and biology and ecosystems; microplastics have not yet been included as part of this global, multidisciplinary coordination effort. In response to a growing public awareness and societal need for better information on plastic contaminants in the ocean, GOOS proposes to develop an observing strategy utilizing relevant globally- and nationally-coordinated in-situ assets. As a first step we propose to bring together technical experts leading individual global observing networks with the leading authorities focused on marine plastic contamination, including international expert groups such as GESAMP, intergovernmental agencies such as UN Environment and other global initiatives involved in marine plastics monitoring, sensor development, and ecosystem impact assessments. The ultimate goal of such formed Task Team would be to establish global coordination of sustained observations of marine plastic contaminants as a Human Pressure Variable which with time might be deemed essential. To this end, the Task Team would implement the concepts from the Framework for Ocean Observing across its three pillars: requirement setting processes, observing capabilities, and data and information product management. Building upon an initial comprehensive readiness level assessment, the Task Team would have the expertise to provide recommendations on how to measure plastic contaminants through a globally accepted sampling protocol and best practices, and on how to ensure the delivery of high-quality information products responding to the societal needs.

\*Speaker

Keywords: global ocean observing system, essential ocean variables, observing strategy, in, situ

## Plastic pollution from a Civic Ecology perspective: A Lanzarote case study

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This study improves the understanding of the current situation of plastic pollution in Lanzarote through (i) diagnosing the local situation; (ii) analyzing stakeholder dynamics; and (iii) engaging the dialectics between local, regional and global dimensions of the issue. Rooted in civic ecology, this ethnographic case study draws on nine years of participant observation and observant participation, along with interviews conducted with 31 participants in 2018, to inform the diagnosis of the local situation and stakeholder dynamics, through a modified grounded theory analysis of the corpora. This analysis also provides the bedrock for exploring the dialectics at play across and between geographic, temporal, and societal scales related to plastic pollution in Lanzarote. From this study, we offer an overview of the evolution of concern related to plastic pollution in Lanzarote, and propose a baseline for collaborative efforts to intervene in the issue going forward locally in Lanzarote and regionally in the North Atlantic system.

Keywords: Civic Ecology, Marine litter, Plastic Pollution, Action research, Microplastics

\*Speaker

## From the Plastics Strategy to microplastics: the policy framework

Valentina Bertato \* 1

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Plastics are used everywhere and too often they are used only once and then thrown away. Consequences of this unsustainable use are pollution of the environment and loss of a resource valuable for the economy. To address these concerns, the European Commission proposed, in the framework of the Circular Economy Action Plan, a Strategy for Plastics. The strategy focuses on four main areas:

Improving re-use and recycling

Curbing plastic waste and litter

Driving investment and innovation towards circular solutions

Harnessing global action.

To achieve its ambitious objectives, the Plastics Strategy relies on many different pieces of legislation: the Waste legislation, the proposal on Single Use Plastics, the proposal on Port Reception Facilities, the revision of the Drinking Water Directive, the evaluation of the Urban Waste Water Treatment Directive, the Marine Strategy Framework Directive and the restriction of microplastics intentionally added in products and oxo-plastics under the chemicals legislation (REACH). A wide range of regulatory tools are proposed, from restrictive measures (where needed) to development of standards to measure emissions, labelling, Extended Producer Responsibility Schemes. The restriction processes that started under REACH aim to harmonise the various national initiatives launched to address some sources of microplastics. By using all available scientific data, the REACH restriction intends to demonstrate that microplastics presence in the environment causes an unacceptable risk. In this way, it will provide an impulse to other actions, including those to control microplastics unintentionally generated from the use of various products (for example, tyres and clothing) or released in the environment (plastic pellets).

Keywords: Plastic, microplastics, Plastics Strategy, REACH

\*Speaker

## REACH Restriction on intentional uses of microplastics

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REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) is a regulation of the European Union (EU), adopted to improve the protection of human health and the environment from the risks of chemicals. REACH applies to industrial, professional and consumer uses of substances as well as uses of substances in articles (e.g. clothes and electrical appliances). REACH is implemented by the European Chemicals Agency (ECHA), in Helsinki. REACH places the burden of proof on companies who must manage the risks of the substances they manufacture and market in the EU. If the risks cannot be managed then the uses of chemical substances can be

‘restricted’. A restriction can be any condition on the manufacture, placing on the market or use of a substance; including a ban.

ECHA is investigating the need for a restriction on ‘intentional’ uses of microplastics. This investigation will conclude in January 2019. Microplastics are added to a variety of consumer, professional and industrial products in the EU including in cosmetics, detergents, agricultural products, medical and veterinary diagnostic devices, paints, inks and products used in the oil and gas sector. A number of these uses will inevitably lead to the release of microplastics to the environment in the EU. Based on our investigation, ECHA may propose that one or more of these uses are restricted. ECHA is assessing the available information on the hazard and risk posed by microplastics to the environment and human health. The conclusions of this assessment will underpin the need for a restriction. Micro2018 offers a unique opportunity for a draft of this review to be shared with experts in the field to facilitate their comments and feedback prior to finalisation. ECHA would like to explore options for this with the organising committee of the conference.

Keywords: European Chemicals Agency, restriction, intentional uses, microplastics

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## Identification and Quantification of Microfibers and Microparticles via Automated Analysis

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The pollution of the oceans with small plastic particles and fibers (smaller 5 mm) is an emerging topic as they have been found already in the remotest areas of the world. For the analysis of these materials, several steps are necessary, starting with the sampling, work up, and finally chemical identification. Due to the small size of the particles, each step has its own challenges. For the identification, FTIR imaging proved to be a powerful tool allowing the automated analysis of complete filters for microplastic particles. Still, the process was not able to identify microfibers in these studies. We present a novel approach for the measurement of microfibers and -particles within one measurement overcoming these limitations. It allowed the identification and quantification of both types within one dataset by FTIR imaging. Via an improved automated analysis approach based on neural networks the differentiation of fibers and particles was possible (see Figure 1). The applied network was trained with data containing the known type and amount of fibers as well as

with data from reference and environmental samples. Subsequently it was applied on datasets from the effluent of waste water treatment plants to determine the type and number of micro-fibers and -particles. In all of these sam- ples, fibers based on cellulose were found. Further, fibers made from different polymer types like polyethylene, polypropylene, polyamide and polyacrylates were successfully identified and quantified. The collected data showed that we developed a versatile and easily applicable tool for the automated analysis of microfibers and –particles within one measurement allowing an improved harmonization of microplastic research, e. g. for the implementation of standardized operational protocols (SOP).

Keywords: Microfibers, Microplastics, FTIR, spectroscopy, harmonization, analysis

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## Future scenarios of global plastic waste production and disposal

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The accumulation of mismanaged plastic waste (MPW) in the environment is a global grow- ing concern. Knowing with precision where litter is generated is important to target priority areas for the implementation of mitigation policies. In this study, using country-level data on waste management combined with high resolution distributions and long-term projections of population and GDP, we present predictions of global MPW production at ~ 1 km resolution from now to 2060. We estimated between 60 and 99 million metric tonnes (Mt) of MPW were produced globally in 2015. In a business-as-usual scenario, this figure could triple to 155 - 265 Mt/y by 2060. The future MPW load will continue to be disproportionately high in African and Asian continents even in the future years. However, we show that this growth in plastic waste can be reduced if developing economies significantly invest in waste management infrastructures as their GDP grows in the future. Using our predictions, we also demonstrate that the majority of MPW (91%) are transported via watersheds larger than 100 km<sup>2</sup> suggesting that rivers are major pathways for plastic litter to the ocean.

Keywords: plastic waste, mapping, forecast, waste management scenario analysis, river inputs

\*Speaker

## **November 22nd – Panel 18.2: 16h30 - 18h30 (AGH), Panel chaired by Ricardo Beiras.**

### **A long term exposure experiment tests the effects of irregular and spherical microplastics on juvenile blue mussels *Mytilus edulis***

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Monitoring studies find increasing amounts of primary and secondary microplastics in all marine ecosystems. Simultaneously, evidence grows that marine benthic filter feeders take up microplastic particles together with their food frequently, while our knowledge about the possible effects of this ingestion is still small. In addition to possible physical impacts such as clogging and damaging, microplastics can presumably also trigger immune responses. So far, very few experimental approaches investigated the potential effects of microplastics over a longer time period and with relevant concentrations of realistic, i.e. irregularly shaped, plastic material. We are currently investigating the effects of two different microplastics (irregularly shaped particles, 1-60 µm and spherical particles, 40µm) on juvenile individuals of the blue mussel *Mytilus edulis* over the course of 12 months. For this, we use concentrations of 0, 10, 1000, 10 000 and 100 000 particles per liter for irregularly shaped particles and 10, 1000 and 10 000 particles per liter for the spherical particles. We intend to answer two questions: (1) At which particle density and after which time span do effects of microplastics become detectable? (2) Do the effects depend on the shape of the microplastic? We will present first results and will discuss the strengths and weaknesses of our approach.

Keywords: filter feeder, biological effect, longterm experiment, mussel, microplastic

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# Differential responses between two marine bivalves to environmentally relevant concentrations of microplastics

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Occurrence of plastic debris < 5mm called microplastics (MPs) mainly resulting from macroplastic's fragmentation has been reported in aquatic ecosystems. Reports have identified MPs and studied their effects on marine organisms. However, there is a lack of information on their potential toxicity at environmentally relevant concentrations. The aim of our study was to compare the accumulation and toxicity of polypropylene (PP) and polyethylene (PE) fragments towards two filter-feeding bivalves: the blue mussel *Mytilus edulis* and the pacific oyster *Crassostrea gigas*. These polymers were selected according to a previous study conducted in situ in the Region of Pays de la Loire (France). Bivalves were exposed in laboratory during 10 days at two environmentally relevant concentrations: 0.008 and 10 µg/L, and to a higher concentration of 100 µg/L of each MPs type, followed by 10 days of depuration in clean seawater. MPs were prepared by cryomilling commercially available products, characterized in terms of size, shapes and number of particles. After exposures, bivalve tissues and biodeposits were chemically digested with KOH 10% and MPs were identified by infrared micro-spectroscopy. Toxic effects were evaluated through the measurement of markers of detoxification, oxidative stress, immunotoxicity and DNA damage. Results showed the presence of MPs in digestive glands of mussels exposed to 100 µg/L of MPs and in biodeposits at all tested concentrations in both bivalves, confirming ingestion of MPs. In mussels, significant increases in antioxidant enzymes were observed at all tested concentrations with differential responses between digestive glands and gills. These responses could be indicative of an oxidative stress. Furthermore, at the same tested concentrations, no toxic impacts were measured in oysters. Overall, this study suggests differential responses to MPs exposition, and reduced toxicity in two filter-feeding bivalves exposed at environmental concentrations.

Keywords: Microplastics, polyethylene, polypropylene, *Mytilus edulis*, *Crassostrea gigas*, oxidative stress

\*Speaker



## Effects of exposure to microplastics alone and with adsorbed benzo(a)pyrene on marine mussels *M. galloprovincialis* at cell, tissue and physiological levels

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Microplastics (MPs) may facilitate the transfer of hydrophobic pollutants from the ocean to organisms due to their hydrophobicity and relatively large surface area (so-called "Trojan horse effect"). This study examined the effects of exposure to polystyrene MPs of 0.5 and 4.5  $\mu\text{m}$  alone and with sorbed benzo[a]pyrene (BaP) on mussels *Mytilus galloprovincialis* in order to elucidate the effects of MP size and the presence of adsorbed contaminants on the organism. MPs were provided daily, mixed with algae, at 1000 particles/ml/day, during 7 and 26 days. Chemical analysis showed that BaP concentrations in mussels exposed to MPs with adsorbed BaP increased with time (up to 150 times greater than background levels) and that smaller MPs posed an increased hazard in terms of the transfer of adsorbed BaP. In histology, large MPs were abundant in the lumen of stomach, mixed with stomach contents, and in the lumen of digestive tubules (DTs), associated to cell debris. Occasionally they appeared within epithelial cells of the stomach, ducts and DTs and in the connective tissue (Figure 1). In all samples, DT appearance indicated a high digestive activity, confirmed by hexosaminidase histochemistry. Increased effects of MPs+BaP compared to MPs alone were seen in neutral red uptake and catalase activity in hemocytes but not in DNA damage despite the genotoxicity of BaP. An apparent increased effect of smaller MPs on DNA damage was also found. Overall, effects in all treatments increased with exposure time. A general hormetic effect was demonstrated on Scope for Growth across MP treatments. This may be due to a compensatory effect whereby mussels increased their absorption efficiency in order to cope with stress observed in biomarker responses. Further work is required to understand the effects of a variety of plastic type, size, shape combinations together with a wide variety of pollutants.

Keywords: Microplastic size, Adsorbed benzo[a]pyrene, Biomarkers, Scope for Growth, Mussels

\*Speaker

# Furthering the dynamic uptake model for virgin microplastics: application to the blue mussel (*Mytilus edulis*)

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Microplastic pollution of the marine environment has been established worldwide as a major issue: microplastic particles have been detected in almost every aquatic habitat on the planet. Due to their small size, and conspicuous presence in the water column, microplastics are often mistaken for food and ingested by marine organisms. Nonetheless, the mechanisms ruling microplastic ingestion and accumulation in biota are still largely unknown, thus hindering the implementation of a proper microplastic exposure risk assessment. In this study, our previously developed dynamic model of microplastic uptake and excretion for the Mediterranean mussel (*M. galloprovincialis*) was further applied to blue mussels (*M. edulis*), and the results obtained from the two studies were compared, to highlight any specie-specific influence. Blue mussels were individually exposed to virgin polyethylene (PE) microplastics (3-12  $\mu\text{m}$ ). Two treatments were tested at two different microplastic concentrations, both equal or below the pseudofaeces threshold to encourage particle ingestion and avoid saturation. After the particles were spiked in the test vessels, ingestion was monitored for three hours, and samples were taken at 12 time-points (0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180 min); furthermore, excretion was assessed for six days after the end of the ingestion phase, with a total of six sampling timepoints (12, 24, 36, 48, 80, 120 h). When compared to the previous model, the increased number of timepoints and the longer duration of the excretion phase in this study improved the fitting of data, thus increasing the accuracy of the calculated uptake and excretion constants. Moreover, the juxtaposition of results from the two mussel species (*M. edulis* and *M. galloprovincialis*) underlines potential differences in the risk posed by microplastic pollution between two important European marine ecosystems, the North Sea and the Mediterranean basin, hence providing a useful risk assessment tool.

Keywords: uptake, excretion, model, mussel

\*Speaker

# Physiological effects of small micro- and nanoparticles - A comprehensive review

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Enough data about physiological effects of micro- and nanoplastics has been published to benefit from a concise overview, which we present as a literature study. The data point towards increasing toxicity of plastic particles with a) decreasing size, b) longer exposure, and c) larger concentrations. d) Different polymer types in e) different weathering stages through different f) exposure routes cause different effects, with different sensitivity between e) species and their f) developmental stages. With these parameters in mind, some seemingly contradicting results, concerning the absence and presence of effects upon exposure to small plastic particles, can be reconciled. To assess the consistency of these tendencies, we categorize these results according to the role of the particle size, condition, polymer type, exposure duration and concentration and set the spotlight on pitfalls in analysis and reporting.

The conclusions support the currently increasing demand for quantitative chemical analysis data on the occurrence of small micro- and nanoplastics in the environment, biota and people. More comprehensive toxicological tests based on such quantitative data should follow suit, in order to estimate the impact of plastics pollution.

Keywords: Nanoplastics, microplastics, toxicity, particle size

\*Speaker

## Mytilus spp. as sentinel species for marine waterborne microplastic pollution; a case study from the Norwegian environment

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Microplastic (MP) contamination is ubiquitous in the environment and many species worldwide have been found to contain MP. The ecological impact of MP pollution is still unknown, thus

there is an urgent need for more knowledge. One key task is to identify species suitable as sentinels for monitoring in key eco-compartments, such as coastal waters. In Norway, mussels (*Mytilus* spp.) have been monitored for hazardous contaminants through OSPAR since 1981. Norway has the longest coastline in Europe and adding MP to the Norwegian Mussel Watch is therefore important in a European and global context. The present study reports MP data in blue mussels (332 specimens) collected from multiple sites (n=15) spanning the whole Norwegian coastline. MPs and microparticles were detected at all locations, except at one site on the west coast. Among the most surprising findings, mussels from the Barents Sea coastline, in the Finnmark region, contained significantly more MP than mussels from most of the southern part of the country, despite the latter sites were located much closer to major urban areas. Only mussels from a site located very close to Oslo, the capital, contained the levels observed in the remote site far north. An average of 1.5 ( $\pm 2.3$ ) particles ind-1 and 0.97 ( $\pm 2.61$ ) particles w.w. g-1 was found. The most common MPs were *Mytilus* spp. as suitable for MP monitoring in coastal waters. However, some uncertainties remain including mussel size as a confounding factor that may influence ingestion, the role of depuration and other fate related processes, and this call for further research.

Keywords: Biomonitoring, Bivalves, Cellophane, Rubber, Ecotoxicology

\*Speaker

## Transfer of microplastics from the water column to sediments by the suspension-feeder *Mytilus galloprovincialis*

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Microplastics ( $\leq 5$  mm) are ubiquitously distributed in the marine environment. Ingestion of synthetic particles has been reported in laboratory settings for a wide range of marine biota, but to what extent the uptake by aquatic organisms affects the dynamic of microplastics in the marine system has received less attention. Suspension-feeders such as bivalves are known for modulating benthic–pelagic coupling and depositional processes. Here, we tested the hypothesis that *Mytilus galloprovincialis* can act as "aggregator" of microplastic particles filtered out the water column to the sediments via feces and pseudofeces, thus making particles bioavailable for benthic detritus feeders. This process may accelerate the vertical flux and sink of particles that for their physico-chemical properties normally tend to be persistent in the water column. Four replicated sets of living

mussels were exposed for 48 h to environmental relevant concentrations (0.2 mg/l) of fluorescently labelled polyamide fragments (129 and 41  $\mu\text{m}$  median grain size), and their biodeposits were analysed to estimate the amount of synthetic particles accumulated. Measured fluxes were compared to those observed in as many replicated control sets of dead mussel shells, to test whether filter feeding by mussels accelerates the natural sinking flux of particles. Three additional sets of living mussels were used to compare the deposition velocities of fecal pellets of mussels exposed to synthetic particles vs not exposed. Results showed that mussels significantly increase the sinking rates of the synthetic particles that tend to aggregate in the biodeposit thereby becoming bioavailable to other benthic organisms. Moreover, our findings suggested that the presence of microplastic particles may negatively affect the sinking velocities of the fecal pellets altering the vertical flux of organic material to the sediment leading to possible changes in benthic sediment geochemistry and communities.

Keywords: microplastic, uptake, bivalves, mussels, biodeposit

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## **November 23rd**

### **November 23rd – Panel 20.1: 8h30' – 10h (AGH), Panel chaired by Carolin Völker.**

## Speculative ecologies: How to deal yet unpredictable effects of microplastics in the ocean from a social science perspective?

Sven Bergmann \* 1

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1) Speculative Ecologies A new phenomenon has emerged in the ocean: plastic-nature-cultures – new entities, habitats and ecosystems caused by anthropogenic debris. The concepts of micro- and nanoplastics point to rather unknown effects, to the uncanniness of the diffusion of plastics in the environment. From a social anthropologist and STS (science and technology studies) perspective I will discuss what kind of social science is needed to deal with plastics as an emerging ecological entity: How can we make decisions when faced with undecidable effects and uncertainty? How to cope with not knowing how different materials behave in the future?

### 2) Responsibility & Environmental Justice

It is not easy to answer the questions who is concerned by ocean plastic and who is responsible for marine litter when it comes to tiny fragments washed up ashore on remote islands where never a piece of plastic was produced. Thus, questions of spatiality, temporality, politics and justice are complicated. In any case, there is a need to search for new tools to address ecological issues as matters of concern and care that show societal entanglement with the problem and its consequences. I will discuss if concepts like environmental justice and "slow violence" (Nixon 2011) might be useful for politicising marine litter.

3) Challenges for social science and policy While policy makers need matters of fact, in science plastic litter is still an "unformed object" with undecidable effects. However, solving the problem is on the political agenda. For that purpose, end-of-pipe solutions and technological fixes gain huge media attention. But not only state or economic stakeholders are keen of this logic, also environmental activism is pervaded by such ideas that lean on sociotechnical imaginaries such as cleaning up large amounts of ocean plastics – although these kinds of solutions are discussed

controversial in scientific debate.

Keywords: Social Sciences, Speculation, Environmental Justice, Policy, Solutions, Nature/Culture

\*Speaker

## Reducing and eliminating plastic waste via change in social behaviour

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Plastic constitutes a large part of everyday consumption. A picnic or barbecue on the beach will usually involve single-use plastic products like straws, plastic-coated paper plates and cups, food wrappings, water and soda bottles etc. Most of these end up as waste in the marine environment. This waste is broken down over time to become microplastics. Microplastics are eaten by sea animals because of their small size. These animals become ill and eventually die as ingested plastic contains harmful chemicals. In a recent statement, the UK Prime Minister Theresa May stated that plastic waste is one of the greatest environmental challenges facing the world. She further stated that this is why protecting the marine environment is central to the agenda at the Commonwealth heads of government meeting. This is also in line with United Nations Sustainable Development Goal 14 – Life below Water.

One way of tackling this problem is via social change and behaviour in following the principles of three Rs.

**Reduce:** The recent move by Swedish retail giant Ikea to ban all single-use plastic from its stores and restaurants is to be applauded and emulated. The UK Prime Minister has also called on Commonwealth members to crackdown on disposable items, and the UK government is proposing a 25 year plan to improve the environment which includes banning the sale of straws, drink stirrers and plastic cotton buds in order to eliminate avoidable plastic waste by end of 2042.

**Reuse:** A large contributor to plastic waste are shopping bags. On 5 October 2015, the UK government started charging on single-use carrier bags in order to encourage people to reuse bags.

**Recycle:** Where it is considered that plastic can no longer be reused, then they need to be properly recycled rather than lumping together with general waste.

\*Speaker

Keywords: microplastics, marine environment, waste, sustainable, reduce, reuse, recycle

# Ecological risk assessment of microplastics in the marine environment

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The presence of microplastics in the marine environment has been an issue of concern for over a decade now, but the environmental risks of microplastics in marine environments have, to date, not been addressed and quantified. The environmental risk assessment of microplastics presented here quantifies, based on a regulatory framework for assessing environmental risks of pollutants, safe concentrations for the marine pelagic and marine benthic compartment. Above these safe concentrations adverse biological effects are likely to occur. At most locations, the in situ concentrations in the upper pelagic compartment remain below the safe concentration. However, even today, we found a potential risk in sites that are heavily polluted with buoyant microplastics. As human populations continue to grow, and if our dependence on plastic does not change under a business as usual approach, we may expect a steady and substantial increase in microplastic concentrations in both the pelagic and benthic marine environment. Adverse effects of microplastics are to be expected on highly polluted beaches and in coastal ecosystems as of the second half of this century if plastics emissions are not reduced.

Keywords: ecological risk assessment, microplastics, marine plastic debris, risk characterisation

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## Risk perception of microplastics. An explorative study identifying aspects potentially influential in risk perception of microplastics

Bomm Laura \* 1

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Although microplastics result from human activities and concern human societies and natural environments, public risk perception has gained rather little scientific attention from social



scientists. Consequently, this study is of exploratory nature and extends the understanding of public risk perception by examining how risks of microplastics are perceived amongst German citizens. Thereby, it is explored which underlying aspects might influence people's risk perception of microplastics and valuable insights for societal change towards a more conscious consumption of plastics are revealed.

By means of a preliminary literature review aspects important in risk perception research of other environmental risks were identified and incorporated in an interview guide. Subsequently, semi-structured interviews were conducted resulting in a more nuanced understanding of the extent to which preliminary identified as well as newly emerging aspects play a role in people's risk perception of microplastics.

This study identified that microplastics are not exclusively perceived as posing a risk to human health, but that risk perception of microplastics is more complex and multidimensional. Findings show that risk perception of microplastics is based on personal as well as societal risk evaluation. Direct experiences with microplastics are limited, but superficial knowledge and limitedly existing indirect experiences are mainly obtained through external informational influences and especially through traditional mass media as well as digital and online environments. Emotions towards microplastics are rather neutral and negative, but seem to be not only caused by negatively perceived media representation of the issue. Microplastics are not extensively appearing in social offline and online environments and normative responses to microplastics are either not yet established or not yet ubiquitous. Several emerged knowledge gaps were combined with existing literature and theories of other environmental risk perception research into a conceptual framework for further social science research, and implications of results for policy and communication are discussed.

\*Speaker

Keywords: microplastics, risk perception, risk communication, social behaviour, qualitative content analysis

## A risk in the making – discussions on microplastics in science, media and public

Carolin Völker \*† 1, Johanna Kramm‡ 1

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While plastic has been known as a factor for environmental pollution – symbolized by the plastic bag – for a long time, recent scientific evidence on the massive accumulation in the oceans and the potential environmental risks associated with microplastics and chemical additives have led to an upswing of the debate. Although the number of studies on the occurrence and effects of

microplastics has risen exponentially, the question of whether microplastics actually pose a risk to the environment could not yet be answered conclusively. In laboratory studies, biological effects have so far only been detected at high particle concentrations with little environmental relevance. Thus, the knowledge about the risks associated with microplastics for the environment and human health is still limited. However, the great mobilization potential of the perceived threat proved to be important drivers for risk management: In 2015, the Microbead-Free Waters Act was passed banning microbeads from rinse-off cosmetics in the USA. For the presentation, we scrutinize how the media have covered scientific studies and in which way ignorance, risk and uncertainty is communicated to the public. We will present results from the media analysis and discuss them in relation to the scientific data as well as to the risk perception of interviewees. We are part of an interdisciplinary group researching plastics in the environment from a social- ecological risk perspective ("PlastX"). Our team comprises researchers from ecotoxicology, chemistry, geography and sociology analyzing plastics from different environmental as well as societal perspectives.

Keywords: Microplastics, risk perception, media analysis, social, ecological risk research

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## “An invisible threat” – Representations of microplastics in the German media

Sarah Schönbauer \* 1, Ruth Müller † 1

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A growing number of scientific studies explore the presence of microplastic particles in the environment, discussing them as a potential hazard for aquatic environments and human health. At the same time, the media has increased its attention towards microplastics, resulting in a growing number of media articles. In this paper, we analyze how the German print media reports about microplastics, how and why it is understood and depicted as a problem and for whom, and which kinds of possible solutions are envisioned. We focus on articles about microplastics in the German daily and weekly high-circulation newspapers that have appeared between 2004 and 2018. This timeframe allows us to trace how the reporting on plastic particles has evolved over time in a specific national context. We show that within this time period, the problem of microplastics has been reframed from a far away problem that primarily concerns other world regions to being understood as a local problem that affects Germany in multiple ways. The discourse about microplastic particles has markedly shifted from debating their occurrence in marine ecosystems towards discussing their presence in local drinking water, streams or food sources. Further, we trace

how actor configurations in German media reporting have shifted over time and who is addressed as responsible for developing solutions to the microplastics problem. Here we find that it is particularly individual consumers who are rendered responsible to reduce the entry of microplastics in the environment, while the producers of plastic products are less frequently addressed. This analysis allows us to better understand and critically interrogate the public discourse about microplastics in the specific German context as one important factor that comes to shape in science funding initiatives and policy actions in the face of a growing public awareness of the presence of microplastics in human environments.

Keywords: responsibility, media representation, local/global distribution

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## **November 23rd – Panel 20.2: 8h30' – 10h (AGH), Panel chaired by TBD**

### **Recycled low-density polyethylene and tyre rubber cause erratic behaviour, oxidative stress and mortality in brackish water copepod *Limnocalanus macrurus* in laboratory experiments**

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Polyethylene from plastic packaging and rubber from tyre wear are key materials polluting the worlds' oceans. Increasing the recycling of post-consumer plastics have been considered one way to reduce the use of fossil fuel for plastic production and the flow of plastic waste to the sea. For example in the European Union the target for 50% recycling of solid municipal waste by 2020 has been set. However, the behaviour of recycled materials in aquatic environments, especially impacts on aquatic organisms have not been enough studied. Our results showed that recycled household post-consumer low-density polyethylene plastic (LDPE) and tyre rubber soaked in seawater cause detrimental effects in copepods. Unwashed LDPE filtrate caused rapid mortality and erratic behaviour even as a 25% dilution. Mortality was most likely caused by organic acids and alcohols arising from the decomposition of food residues in unwashed LDPE food packages. Elevated stress responses were measured also in rubber filtrates. These stress responses were most likely caused by the dissolved zinc and copper. The future studies on potential impacts of marine plastics should thus focus also on recycled materials and waste water discharges from recycling plants.

Keywords: Polyethylene, Postconsumer plastic, tyre rubber, filtrate, detrimental effects, copepods

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# Microplastics alter feeding selectivity and faecal density in the copepod *Calanus helgolandicus*

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Copepods are a key component of temperate marine food webs, constituting a food source for higher trophic levels, and an important link in the transfer of atmospheric carbon from surface waters to the seabed. Microplastic ingestion has been documented in copepods from laboratory and field studies, providing some knowledge on the associated impacts, such as diminished energy allocation, fecundity and survival. Gaps remain regarding whether active or passive selection of microplastic may influence their uptake, that of natural prey, and how microplastic ingestion affects the sinking of egested materials to the seabed. Here, we use experimental studies where algal prey and plastic of similar sizes and shapes were provided at a ratio of 3:2, to test: (1) whether prey selection by the copepod *Calanus helgolandicus* is affected by the size or shape of microplastics and algae they are exposed to; and, (2) whether ingestion of microplastics with different densities alters the vertical flux of copepod faecal pellets through the water column. Exposure to nylon fibres resulted in a 6% decrease in ingestion of similar shaped algae and a 6% increase in ingestion of cylindrical algae. Conversely, exposure to nylon fragments led to a 7% increase in consumption of algae differing in size and shape to the plastic and an 8% decrease in ingestion of algae that was a similar shape and size. Results from the sinking rate study suggest that microplastic ingestion by *C. helgolandicus* alters the sinking velocity of faeces; for example, faeces contaminated with low-density polyethylene sank significantly slower than controls, whilst sinking rates increased when contaminated with high-density polyethylene terephthalate. Our results suggest that in areas where hotspots of microplastic and zooplankton converge, for example in oceanic gyres, microplastic pollution is likely to influence zooplankton prey selection and alter the sinking rates of their faecal pellets.

Keywords: microplastics, uptake, zooplankton, egestion, sinking rates

\*Speaker

# Microplastic Ingestion by Calanoid Copepods in the SE Black Sea

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Microplastic ingestion by two dominant calanoid copepod species *Calanus euxinus* and *Acartia clausi* in the Black Sea was assessed. Zooplankton samples were collected from vertical net tows from 200 m to surface between May 2015- April 2016 as a part of TÜBİTAK 117Y207 project. To assess presence of microplastics, organic tissues were removed by 35 % hydrogen peroxide treatment. Microplastics was visually counted using a binocular microscope and classified (fiber, film, fragments). Microplastics were detected in both the copepods. Twenty-seven plastic particles were detected after digestion of 1192 *Calanus euxinus* resulting in an encounter rate of  $0.032 \pm 0.04$  particles/*Calanus euxinus* (one particle/every 44 *Calanus euxinus*) (Figure 1). For *Acartia clausi*, a total of 9 particles were detected from 1100 individuals resulting in an encounter rate of  $0.012 \pm 0.018$  particles/*Acartia clausi* (one particles/every 122 *Acartia clausi*). The difference in ingestion between the two copepod species was significant (t test,  $p < 0.05$ ). The average ingested size of microplastic particles was greater in *Calanus euxinus* ( $134 \mu\text{m} \pm 176 \mu\text{m}$ ) than *Acartia clausi* ( $57 \mu\text{m} \pm 58 \mu\text{m}$ ). Overall, approximately 68 % of particles ingested were fragments followed by film and fibre. Colour of ingested particles consisted dominantly of black, blue and red. Our results provide first evidence of ingestion of microplastic by two copepod species critical to the Black Sea pelagic food web. This suggests that zooplankton can act as a vector for microplastic transfer to upper trophic levels, including humans.

Keywords: Ingestion, Copepod, Food web, Black Sea

\*Speaker

## Microplastic properties and environmental conditions influence PAH sorption and bioavailability to copepods

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Many marine species ingest microplastic (MP), exhibiting extended periods of retention in some cases. Organic pollutants associated with MP may present an alternative exposure route for these chemicals to marine species. However, the effect of PAH sorption to MP on PAH bioavailability remains poorly understood. Here, we investigate sorption kinetics and present adsorption isotherms for two PAHs (fluoranthene and phenanthrene) to MP in natural seawater at 10 and 20 °C. Spherical polyethylene (PE) and polystyrene (PS) microbeads of 10  $\mu\text{m}$  and 100  $\mu\text{m}$  were used, representing ingestible and non-ingestible particle sizes for copepod species used in the bioavailability studies. Linear, Freundlich, Langmuir, Dual Langmuir, Redlich-Peterson and Dubinin-Ashtakhov isotherms were fitted to the data. For polymer mass and particle surface area, PAH sorption increased in the order PE-10 > PS-10 > PE-100. For PS-10 and PE-10 at 10°C, the Redlich-Peterson isotherm model best described the sorption, indicating a combination of monolayer and multilayer adsorption. For PE-100, linear isotherms fitted best, suggesting that sorption is influenced by absorption. For PE-10 at 20 °C, the Dubinin-Ashtakhov model fitted best, indicating higher temperatures facilitate PAH transitioning into micropores. Using a novel approach (Figure 1), the modular effect of PAH sorption to PE-10 and PE-100 on PAH lethality and accumulation was investigated using two marine copepod species (*Acartia tonsa* and *Calanus finmarchicus*). The freely dissolved fraction of PAHs ( $C_{\text{free}}$ ) was measured before and after exposure, and the chemical body burden ( $C_{\text{finmarchicus}}$ ) and observed lethality (both species) were used to determine PAH bioavailability. PE-10 reduced  $C_{\text{free}}$  by ~ 50 % (FLA and PHE), PE-100 reduced  $C_{\text{free}}$  by 21±5 % (FLA) and 32±3 % (PHE). The difference in  $C_{\text{free}}$  reduction was not reflected in a reduction of lethality and body burden (> 50 % for both MPs), indicating a negligible amount of MP-sorbed PAH is bioavailable.

Keywords: Microplastic, PAH, sorption, bioavailability, toxicity

\*Speaker

## Benchmarking the uptake and excretion dynamics of microplastics in the marine copepod *Calanus finmarchicus*

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The marine copepod *Calanus finmarchicus* is a filter feeder and a keystone species of the Northern Atlantic that, alongside other crustaceans, is known to ingest microplastic (MP). Potential effects of MP ingestion include obstruction, abrasion, lower energy budget due to reduced food intake, and toxicity related to the leakage of chemicals from microplastics into the organism. The magnitude of all these effects is largely determined by microplastic uptake and excretion rates, and particularly by retention or accumulation times inside an organism. However quantitative uptake and excretion data are currently unavailable. In the current study, non-ovulating female *C. finmarchicus* were exposed 0-96 h to a non-restrictive concentration (750 MP particles/mL) of a comprehensively characterized 10 mm spherical polystyrene MP in the presence of microalgae (7500 cells/mL). Excretion dynamics were studied over 1-72 h after a 24 h exposure to MP in either the presence or absence of microalgae. Custom made transparent plankton wheels immersed in a water bath were used to expose *C. finmarchicus* in a semi-static setup to keep MP and microalgal particles in suspension and to maintain constant temperature. MP counts were performed in triplicate in exposure media, copepods and their fecal pellets as well as on container walls for a total of 16 sampling time points. Findings show unconventional uptake kinetics for MP in *C. finmarchicus* when compared to dissolved organic chemicals. Excretion of MP was rapid although there was indication that retention may occur. Excretion dynamics were affected by the presence of microalgae. The current study was designed as a benchmarking exercise with commercial microbeads of uniform size. The findings can therefore not be generalized for MPs of other shapes, compositions, conditions, densities and sizes, but should rather be considered as a reference for future studies using MP with more environmentally relevant characteristics and concentrations.

Keywords: uptake/excretion/retention/benchmarking

\*Speaker

## Are fish that live in different zones reliable indicators of microplastic pollution?

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Ingestion of microplastics (MPs) by fish has been widely demonstrated, although the capacity of MPs to affect the ecology of fish is still to be understood. The aim of this study



is to further investigate the uptake of MPs by species which are environmentally exposed to different habitats and with different feeding behaviours. Therefore, we compare the MPs inherent characteristics that affect their distribution in the water column and the behaviour of fish. The species: *Boops boops* and *Spicara smaris* have been chosen since they favour a midwater layer in the water column; *Oblada melanura* and *Scomber scombrus* are generally found close to the surface; *Mullus barbatus* and *Merluccius merluccius* are benthic species which are found over soft sediments. A minimum number of 30 individuals for each species was collected locally from Sardinia (Italy) and afterwards weighed and measured. The size classes are taken into consideration in order to note if the life stages are affected in different ways by MPs. The detected MPs are divided into typologies, size classes, morphology and colour, while the polymer type is determined by FT-IR analysis. The results from this work can add useful information to show the relationship between the ingestion of MPs and the ecology of fish and eventually take one small step to understand the reasons for the ingestion. Moreover, this research could benefit the identification of appropriate indicators for the presence of MPs in the Mediterranean Sea.

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## **November 23rd – Panel 21.1: 10h30' - 12h30' (AGH), Panel chaired by Martin Wagner.**

### **Bioavailability of microplastic to marine zooplankton: effect of shape and infochemicals**

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The ingestion of microplastics has been recorded in several marine zooplankton taxa. However, the underlying mechanisms that influence plastic ingestion remain poorly understood. We investigate how shape (bead, fibre and fragment), in combination with the infochemicals dimethyl sulfide (DMS) and dimethylsulfoniopropionate (DMSP), affect the ingestion rate of microplastics in species of zooplankton. Laboratory studies typically use pristine microplastics of uniform size, shape and colour, which is not representative of those found in the environment. Whilst microplastic beads are used for the majority of laboratory experiments, field studies report microfibrils to be the most abundant plastic particle in the environment, and yet it is unclear which shape has the highest bioavailability to or impact on zooplankton. There is growing evidence that mechanisms such as chemosensory cues could influence bioavailability of microplastic via adsorption of chemicals present in the environment. One such chemical is DMS, a bacterio- and phytoplankton-derived marine trace gas. Research has shown that Calanoid copepods elicit foraging behaviour in the presence of DMS. It is possible that DMS, along with other chemicals such as DMSP, could be adsorbed to the surface of the microplastic potentially increasing the palatability of the plastic. Zooplankton is a crucial food source for many secondary consumers, consequently this represents a route whereby microplastics could enter the food web and transfer up the trophic levels. Additionally many species of shell- and fin-fish have a larval stage in the zooplankton. Therefore it is important to understand which factors can influence the bioavailability of microplastics to zooplankton as there may be implications for higher trophic levels and fisheries.

Keywords: Marine zooplankton, microplastics, selectivity, bioavailability

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# The translocation of microplastics to lipid droplets of *Daphnia magna* is an artefact

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The last decade has seen a surge in research investigating various aspects of micro- and nanoplastics, including occurrence, uptake, and potential effects in biota. Our knowledge about biota-particle-interactions is still limited and often based on early studies that – due to the infancy of the field – may have deficiencies in the experimental design and quality controls. One such example relates to the potential of plastic particles to cross the epithelium of the digestive tract and translocate to other tissues. This has been reported in the literature for the freshwater cladoceran *Daphnia magna* and – if true – is toxicologically relevant. The aim of our study was to replicate previous findings while covering additional experimental scenarios. We exposed 205 starved *Daphnia magna* neonates to two particle types (fluorescent polystyrene spheres of 20 nm and 1000 nm diameter) at two concentrations (2 µg/L and 2 mg/L) for two time periods (4 h and 24 h). Additionally, we adapted a tissue clearing method to the use with *Daphnia* to improve visibility of particles inside the specimen. We used confocal laser scanning microscopy to investigate the translocation of particles from the gut to other tissues, including lipid storage droplets. This is supplemented by experiments regarding the leaching of the dye from the particles. The 1000 nm particles can be imaged individually inside the specimen. At the higher particle concentration we detected fluorescence in the lipid droplets that for the 1000 nm particles was clearly separate from particles and faded within minutes of observation. Our findings indicate that tissue translocation to the lipid storage droplets reported previously is probably an experimental artefact caused by the leaching of the dye used in commercially available plastic beads. This underlines the importance of scepticism and replication in our field, especially if a plausible underlying mechanism is lacking.

Keywords: microplastics, nanoplastics, tissue translocation, Cladocerans, Zooplankton, *Daphnia*, CLSM, fluorescence

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## Effects of low density polyethylene microplastics contaminated with PFOS on *Daphnia magna*

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Low density polyethylene microplastics (MPs) are widely dispersed in the aquatic environment. In several ecosystems, they may be contaminated with perfluorooctane sulfonate (PFOS) due to the incorporation of this chemical during the manufacture of MPs or during their presence in the environment. Therefore, it is of interest to assess the effects of MPs contaminated with PFOS on the biota. In acute and chronic bioassays, *Daphnia magna* was exposed to MPs (125 - 500 µm) contaminated and not contaminated with PFOS and the effects were assessed, using mortality (acute bioassays) and several reproductive parameters (chronic bioassays). MPs alone and contaminated with PFOS did not induce significant acute effects in the range of concentrations tested. Tests to determine the reproductive effects will be discussed in relation to the properties of the MPs tested and their contamination by PFOS.

Keywords: microplastics, LDPE, *Daphnia magna*, freshwater, PFOS

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## Effect of chemical and biological ageing on polyethylene and the subsequent toxicity in *D. magna*

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Microplastics have been found ubiquitously across the globe, in studies ranging from the deep ocean trenches to arctic sea ice to freshwater streams. Following this, there has been increasing societal pressure and scientific interest, leading to calls for more robust scientific research and interdisciplinary discussions, and consequent led legislation and policy changes. Micro, and more recently nano, plastics have been found to cause a range of detrimental effects on organisms, which are usually dose dependent. These effects have been shown to impact feeding, reproduction and behaviour, which ultimately affects the survival rate of the organisms. However, in many cases the environmental realism or dispersion characteristics of the plastics have been quite poor. This study aims to look at the effect of chemical and biological ageing on micro and nano plastics, and

subsequent impacts on the test organism *Daphnia magna* (a keystone species) under a range of field and laboratory test scenarios. The goal is to further elucidate the role of environmental realism in the studies, such as the role of natural organic matter in the medium. Corona formation on plastic particles changes the surface characteristics which can affect subsequent biological and chemical interactions. In this study we conditioned and aged polyethylene spheres in both natural waters collected along an urban-rural gradient in Birmingham, UK, and in artificial river waters synthesised in the laboratory, to compare the difference that biological and chemical ageing could have on pristine polyethylene spheres. We characterised and imaged plastics at various stages throughout the ageing process to assess agglomeration and dispersal compared to the ageing in the test medium. Aged plastics were then exposed to *Daphnia magna* to assess if there is any significant difference between the conditioning methods on the resulting uptake, retention and any subsequent effects from the polyethylene micro and nanoplastics.

Keywords: Toxicity, *Daphnia magna*, Polyethylene, aged plastics

\*Speaker

## Microplastics-Daphnia interactions: importance of surfactant

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Possible interactions between microplastics and aquatic organisms are complex and not completely understood. Most studies are realized using polymeric microbeads with associated surfactants, which are necessary to insure their stability and the role of the surfactant in the interaction has to be investigated. In this study, polyethylene (PE) small microplastics (between 0.8 to 3  $\mu\text{m}$  in size) were synthesized by micro-emulsification without any surfactant and with different surfactants (Tween 60 and a biosurfactant from microalgae exudates). *Daphnia magna*, an aquatic primary consumer, which is an ecotoxicological study organism, were exposed to these particles in order to evaluate their potential acute toxicity and monitor interaction. Expositions were run at different concentrations (1, 30, 60 mg/L) of the three kinds of particles and with or without feeding on short-term exposure with 30 organisms per condition. Following 72 hours exposure, no microparticles ingestion by *D. magna* could be observed for control and Tween 60 PE assays and

only few particles were visualized in most of individuals in interaction with uncoated PE. Great quantities of microparticles were detected in digestive track of all animals exposed to PE coated with biosurfactant leading to immobilization and mortality rates higher than in controls. After one week, at high concentration, reproduction was also impacted for biosurfactant and Tween conditions.

These first results underline the importance of surfactant effect on microparticles ingestion by aquatic organisms. No ingestion of MP PE coated with Tween should be explain by the capacity of *Daphnia* to taste microparticles with gustative chemoreceptor (Peñalva-Arana et al., 2009). In conclusion, model microplastics should be designed with surfactant of environmental relevance to assess toxicity on aquatic organisms.

Peñalva-Arana, D. C., Lynch, M., & Robertson, H. M. (2009). The chemoreceptor genes of the waterflea *Daphnia pulex*: Many Grs but no Ors. *BMC Evolutionary Biology*, 9(1), 8–11.

Keywords: Plankton, surfactants, ingestion

\*Speaker

## Exposure to conventional but not biodegradable microplastics decreases fitness in *Daphnia magna*

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Conventional oil-based polymers are the major source of microplastic pollution, whereas contribution of biodegradable polymers (bioplastics) is considered negligible. However, as production of bioplastics is increasing, whereas their biodegradation under ambient conditions is relatively slow, it is likely that their contribution to plastic waste will increase and become comparable to that of the oil-based plastics. Therefore, it is important to understand potential environmental impacts of both polymer types. We compared effects of exposure to polylactic acid (PLA; bioplastic) and polystyrene (PS; oil-based polymer) on primary life history traits in the crustacean *Daphnia magna*, a standard model species in ecotoxicology. To exclude particle effects caused by food dilution and thus identify microplastic-specific effects, kaolin clay was used as a reference treatment. In total, four treatments were included: PLA, PS, clay (reference), and control (food only). The exposure was conducted over 21 d using a plankton wheel to keep test particles and algae in suspension for comparable exposure concentrations. In the PS treatment, we observed high mortality, decreased feeding rate and reproductive output compared to all other treatments. These

effects were not caused by toxic monomers of styrene or additives leaching out of the polymer, which was demonstrated in a follow-up test with the PS leachates. By contrast, no significant effects were found in the daphnids exposed to PLA compared to the reference treatment. Thus, a significantly higher toxicity of the conventional polymer was observed, whereas effects of the biodegradable microplastics were similar to those caused by the ubiquitously occurring clay particles. More studies are needed to identify the mechanisms of PS toxicity and to confirm the observed ecotoxicological differences between the polymer types using different test materials and natural particulates.

Keywords: bioplastics, benchmarking, *Daphnia magna*, ecotoxicity testing, reference materials

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## Towards improved predictions of dispersal of weathering microplastics in the aquatic environment by numerical modelling

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The distribution and quantification of microplastics (MP) in the aquatic environment remains very difficult. Numerical models are a useful tool for the assessment of the dispersion of MP particles in water bodies. Thus far, the prediction of the fate of MP in aquatic systems has been carried out by models for passive tracer dispersal, which may help to find possible pathways from source to accumulation spots. But these models do not account for many processes and do not predict particle concentrations. Thus far, several studies have been able to find approximate predictors for the terminal settling velocity of individual particles, but since the size and shape of different particles varies from particle to particle, it is not straightforward to replace each particle by a single representative particle. The proposed model assumes that the MP particles around a certain point location in the model show a lognormal particle size distribution (PSD). It is demonstrated that the net settling flux of such a particle population cannot be reproduced by the settling flux of the same number of particles with the mean size of the entire population, but is dominated by the heavier particles. Since the latter settle down faster, the PSD changes in space and time: with

increasing time and drift from the source the PSD at the water surface is expected to shift its mean size to smaller fractions, while the PSD will show a relatively larger mean size with increasing depth. Besides this differential settling, the particle size and density is also expected to change due to weathering, biofouling and associated aggregation. Within the framework of the WEATHER-MIC project it is the ambition to develop an improved model, based on the methodology adopted in cohesive sediment transport studies, and to demonstrate its potential with applications to some Scandinavian coastal areas.

Keywords: settling velocity, weathering, biofouling, population kinetics, dispersion modelling

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## Microplastics Disrupting the Biological Pump?

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## **November 23rd – Panel 21.2: 10h30' – 12h30' (AGH), Panel chaired by Gunnar Gerdts.**

### **Microplastics concentrations in freshwater during a flood event, a case study of the Seine river catchment**

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Despite recent research efforts, many questions still exist about sources of microplastics, especially their transport in freshwater from land to sea. Particularly, the consequences of flood events on microplastics transport are almost unknown although recent papers mention that large quantities could be transferred. A flood event occurred in January 2018 on the Seine river catchment. With a surface area of approximately 75 000 km<sup>2</sup>, the Seine basin is a densely populated area (16 million inhabitants mainly clustered in Paris metropolitan area). During the three week flood event the microplastic concentrations were assessed in the Seine and compared to low flow period concentrations. Three different sampling sites were monitored: one 100 km upstream Paris (called "Upstream" in the figure) and two others ("Downstream 1" and "Downstream 2") 20 km and 45 km downstream. Three sampling campaigns have been conducted, one before the peak flow and two after. A 300 µm net and a 80 µm net were used. The 300 µm net was dropped directly in the middle of the river for 5 minutes while 20 L of freshwater was collected and filtered in the 80 µm net. The samples were sieved and digested with a H<sub>2</sub>O<sub>2</sub> 30 %. The samples were then filtered and microplastics were counted with a stereomicroscope coupled with an image analysis software. The fragments and a part of the fibers (30% of the total fibers) were analyzed with FTIR spectroscopy. Work is in progress. The microplastics in the 1 mm to 5 mm size range, collected with the 300 µm net, were counted and observed. Highest concentrations are found before the peak discharge for all sites and concentrations can be 5 to 10 times lower after this peak. Previous works found 0.54

fragments/m<sup>3</sup> in Paris during low flow periods, the flood event could provoke microplastics resuspension.

Keywords: Microplastic, flood event, digestion, spectroscopy FTIR

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## Seasonal Patterns of Microplastic Occurrence and Affiliated Bacterial Communities

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While there exists now a considerable sum of knowledge on marine microplastics and their associated microbial communities, still very little is understood of their kinetics within the vast marine ecosystem. In this study, we aim to better understand the underlying dynamics of microplastics and their association with bacteria in the surface waters of Helgoland Roads (North Sea) coupled to seasonality and environmental conditions as potential driving forces. Regular monitoring of these microplastics and their bacterial colonizers is carried out through weekly sample collection and processing over a complete seasonal cycle. In order to facilitate this, a novel, rapid processing pipeline was designed, adhering to a maximum 5-day timeline, and employed to obtain a high resolution dataset otherwise infeasible via traditional processing methods. Through spectroscopic and molecular approaches, microplastics found are characterized and quantified, and the composition patterns of their associated bacterial communities analyzed. Data obtained will then be compared to the temporal datasets from the Helgoland time-series consisting of physicochemical conditions that have been measured weekly over the last 5 decades. Through this work, we hope to establish a more comprehensive understanding of the mechanics behind microplastic occurrence, the compositional behaviour of MP-colonizing bacteria, and how physicochemical conditions potentially dictate these patterns. Simultaneously, the rapid processing pipeline will serve to lubricate the implementation of more marine monitoring programs as called for in the European Union's Marine Strategy Framework Directive.

Keywords: Microplastics, microbial biofilms, microplastic monitoring

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# Characterization of microplastics from sea turtles (*Caretta caretta*) stranded along the northern adriatic sea

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The Northern Adriatic Sea represents one of most critical areas in the Mediterranean for human-turtles interaction. Loggerhead sea turtles (*Caretta caretta*) found dead along the Veneto coast were collected from the University of Padua in 2016 and post-mortem examinations were performed according to international guidelines. During necropsy, stomach of 16 turtles in good conservation status has been collected for identification and characterization of plastics contents. Macro and micro-plastic substances were extracted and examined using a recently validated, standardized protocol (Avio et al., 2015) based on trituration of dried samples, separation under density gradient and filtration under vacuum, partial digestion in 15% H<sub>2</sub>O<sub>2</sub>, visual sorting and iFT-IR characterization. Extracted particles were microscopically observed, photographed, measured at their largest cross section, and categorized according to both size classes and shapes.

Only one macroplastic particle has been found in one turtle; while microplastic particles have been detected in six specimens (one for animal). The most common sizes classes ranged between 1 and 5 mm (57.1%), while none particles smaller than 0.1 mm have been detected. The shape of plastic particles extracted was largely dominated by lines (43%), followed by films (29%), fragments (14%), and pellet (14%). iFT-IR analyses indicated that polyethylene was the most common polymer, contributing to 43% of all the particles isolated. In this study, the microplastic contents in sea turtles stranded along the North-Adriatic coast has been characterized for the first time. Overall, the 44% of the investigated animals presented plastic elements in their stomach (1 items for each one) and just one ingested a macroplastic fragments representing a lower number compared to that obtained during investigations on turtles coming from other basins. Further investigations (including higher number of organisms and investigation on entire gastrointestinal tracts) are needed to clarify the trend of plastic ingestion in the Adriatic sea turtles.

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Keywords: Sea turtle, microplastic, Adriatic sea

# Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean

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More than 5.25 trillion of plastic pieces have been estimated to be floating at the sea surface. Plastic marine debris on beaches and floating in seawater is exposed to solar UV radiation undergoing weathering degradation. It can develop surface cracks and fragment into progressively smaller particles reaching microscopic sizes (< 5 mm, microplastics). Detrimental effects of plastics on marine organisms at different trophic levels have been widely reported, making plastic pollution a global environmental concern. However, the impacts of plastic debris on the lowest trophic levels, such as the microbial food web, remain enigmatic. Plastic is known to leach organic compounds to the aquatic media. The smaller the piece, the higher its surface to volume ratio and its potential for leaching. However, the contribution of plastic leaching to the dissolved organic carbon (DOC) pool in the ocean and its impact on the lowest trophic levels, such as the microbial food web, is still unknown. In this work we present experimental evidence that plastics release dissolved organic carbon (DOC) into the ambient seawater stimulating the activity of heterotrophic microbes. It is predicted that plastic waste entering the ocean will increase by 10-fold over the next decade, resulting in an increase in plastic-derived DOC that could have unaccounted consequences for the activity of marine microbes and for the ecosystem.

Keywords: Marine microplastics, dissolved organic carbon, marine microbes

\*Speaker

# Plastic intake in mediterranean sea birds

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As in most marine organisms, seabirds all around the world are accidentally ingesting plastic, putting them at risk of intoxication, ulcers, blockage of the gastrointestinal track and internal injuries. However, there are scarce evidences in the Mediterranean. For this reason, we have

examined the stomach content and determined the number of fragments with plastic appearance of 390 birds from 10 different species caught by longliners by accident mainly in the Balearic Sea. Next, we have characterized the obtained fragments' size, weight and colour, as well as whether they are plastics or not, and what kind, in a subsample of them (930), using an infrared spectrometer. The Scopoli's shearwater (*Calonectris diomedea*) has shown the highest plastic intake (80,7%, 1106 fragments), followed by the Mediterranean shearwater (*Puffinus yelkouan*, 56,9%; 169 fragments) and the Balearic shearwater (*Puffinus mauretanicus*, 46,9%; 133 fragments). Gulls and gannets showed a far inferior intake frequency (*Chroicocephalus ridibundus* 0%; *Morus bassanus* 5,3%; 1 fragment; *Ichthyaetus audouinii* 6,7%; 10 fragments; *Larus michahellis* 13,6%; 13 fragments). The colour analysis shows a possible active selection of black coloured fragments (46% of the analysed fragments). The infrared spectrometer analysis has revealed that 65 of these fragments have a composition different from that of plastic. The rest have been confirmed as plastics, mostly polyethylene (between 69 and 82%), followed by polypropylene and other plastics. These results confirm that plastic fragments are being ingested by mediterranean seabirds in worrying percentages, specially considering that the most affected are the three endemic mediterranean shearwater species, currently in decline for several reasons.

Keywords: sea birds, mediterranean, characterization of plastic fragments, plastic intake, necropsies

\*Speaker

## Evaluating the effect of plastics on marine microbial communities

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Plastic debris is now ubiquitous in global oceans and if left unchecked the problem will increase in line with rising global production and consumption. To learn about the unaccounted consequences of the plastic debris in the ocean, it is crucial to understand how it influences the microbial communities in marine environment that play key roles in ecosystem functioning e. g. supporting marine food webs and driving major elemental cycles. Research has shown that microbes readily colonise marine plastic debris and community members of these microbiotas are speculated to have toxic, pathogenic or plastic degrading-species. However, the role of plastic in selecting for unique microbial communities is largely unknown, as is the influence of colonising microorganisms

on the fate of plastic waste. Our study focused on determining the influence of plastic types on the structure and diversity of seawater bacterial communities under laboratory conditions. A microcosm test method that simulated the environmental conditions (temp = 10 oC, humidity = 80%) of marine ecosystem was undertaken over a period of 22 days. High-throughput 16S amplicon sequencing and community fingerprinting were used to determine the effect of plastics on the microbial communities from natural seawater. Communities were compared with respect to plastic treatments and physico-chemical properties of the marine environment to determine the factors influencing the bacterial communities. Preliminary data demonstrated a significant effect of the plastics on the microbial community diversity, with the presence of plastics altering key abiotic parameters of the seawater.

Keywords: plastics, marine bacteria, community based bioassessment, molecular ecology

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## Microplastic presence in loggerhead turtles (*Caretta caretta*) stranded in Gran Canaria Island.

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The European Project INDICIT had proposed sea turtles as marine debris indicator on the European waters. In order to determinate microplastic ingestion by sea turtles, INDICIT project had standardised methodologies for sampling and conducting shared analyses. Aim of this study was to determinate microplastic ingestion by loggerhead turtles stranded in Gran Canaria Island, both dead and alive. For dead turtles, necropsies were carried out and complete digestive track was analysed by sections (oesophagus, stomach and intestine). For stranded live turtles, faeces were sampled daily during the first ten days and 3 times per week up to one month after arriving date. Sampling time was determinate according to the results of transit times experiments carried out in Gran Canaria from 2015 to 2017.

Both, macro and micro-debris were collected according to INDICIT protocols and analysed separately. On micro-debris, number of items and weight of each category was determinate according to INDICIT classification based on the European MSFD. Finally, microplastics abundance was related to sea turtle body size, weight, body condition index and stranding cause. Preliminary results showed 100% microplastics occurrence on sea turtles (dead and alive) stranded

in Gran Canaria Island. These microplastics occurrence is the highest between INDICIT partners, perhaps induced by a most accurate methodology ("2 sieve method"). Final results indicate that sea turtles are effective indicators of marine micro-debris on the ocean, and allow the implementation of adequate action plans for to achieve GES in European waters.

Keywords: Turtles, Canary Islands.

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## COLONIZATION OF MICROPLASTICS AND TRANSFORMATION OF SORBED PCBs BY A MARINE MICROBIAL COMMUNITY

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Microplastics (MPs) have a high probability of ending up in the marine environment, in particular on anaerobic bottom sediments due to their density increase caused by fouling. MPs also act as sinks for hydrophobic persistent organic pollutants, such as polychlorinated biphenyls (PCBs), and vehicle them to the marine food web through ingestion. Little is known about the colonization of pristine and PCB-polluted MPs in the marine environment and the effects of MPs biofilms on sorbed pollutants, especially in marine sediments, where organohalide respiring microorganisms able to mediate the reductive dechlorination of PCBs occur. We thus investigated the colonisation dynamics by an organohalide respiring marine microbial community and its biotransformation activity towards Aroclor 1254 PCBs sorbed on LDPE, PET, PS, PP and PVC pellets (30 mgPCBs/kgMPs) incubated in slurry microcosms of marine sediment suspended in seawater. Reductive dechlorination of PCBs associated to MPs was observed after two weeks of incubation and was faster on PVC and PS, followed by PET, PE and PP. The same dechlorination pattern was observed on all MPs. MPs were rapidly colonized by microbial community, followed by biofilm maturation with no significant differences between MPs contaminated and not. A greater biomass growth was observed on PVC pellets than other MPs. The biofilm community on MPs was not significantly affected at the Phylum level by the type of polymer and the presence of the PCBs; however, the relative abundance of Dehalococcoidia, i.e., of the organohalide respiring Chloroflexi members of the community, was remarkably increased on PCBs-contaminated MPs. These data suggest that MPs contamination can favor the enrichment of degraders in biofilm; microbial colonization of contaminated MPs might therefore contribute to their partial detoxification. The

financial support by the Italian Ministry for Education, University and Research through the JPI-Oceans project "Plastox" is greatly appreciated.

Keywords: microplastics colonization, bacterial community, persistent organic pollutants, biodegradation, organohalide respiration

\*Speaker



## **November 23rd – Panel 22.1: 14h30' – 16h30' (AGH), Panel chaired by Arnaud Huvet.**

### **Nanoplastics exposures to gametes and embryos affect larval development of oyster**

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The Pacific oyster *Crassostrea gigas* represents one of the most important shellfish resources and plays an important role in estuarine and coastal marine habitats, characterized by intense anthropogenic pressures. As they achieved an external fertilization for reproduction, early life stages must face environmental stressors such as plastic debris. Mismanagement of plastic waste by human societies leads to a significant release of plastic into the oceans, which then fragments into small particles including micro- (MP < 5 mm) and supposedly nanoplastics (NP < 100 nm). Here, we investigated the toxicity of the amino-nanopolystyrene (50 nm) on gametes and embryos at a concentration of 0.1 µg.mL<sup>-1</sup>. Cross-exposures on spermatozoa or/and oocytes were performed followed by exposure or not at the embryonic stages (all in all, 8 conditions). Exposures of gametes caused no effect on fertilization. In contrast, exposures of embryos induced a decrease in the normal D-larval yield and affect the entire larval development - larvae was not exposed - characterized by a settlement delay of one day compared to the control condition. Several cellular endpoints were measured on gametes during exposures (cell viability and reactive oxygen species in female and male gametes; mitochondrial membrane potential in spermatozoa) and on larvae (lipid index, transcriptomics analysis) that will be discussed at the MICRO conference.

Keywords: Nanoplastics; oyster; larvae; gametes

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# Eat microplastics, die early? Do microplastics lead to higher mortality of barnacle larvae

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Plastic litter negatively impact the marine life. Plastic debris smaller than 5 mm in diameter are classified as microplastics. Microplastics can be generated during plastic manufacturing or came from degradation of larger plastic fragments in the natural environment. Compared to macroplastics, the influence of microplastics on marine life are still largely unknown. Although most studies have evaluated the effect of microplastics on responses of adults but relatively less studies focus on larvae. Another constraint is the short experiment time. In the present study, we used barnacle naupliar larvae (*Amphibalanus amphitrite*), as a model organism for crustacean larvae, to investigate the ingestion of four sizes of polystyrene microbeads (diameter 1.0, 6.8, 10, 20  $\mu\text{m}$  respectively) at four concentrations (1000, 100, 10, 1, 0 beads  $\text{ml}^{-1}$ ). After exposing to microplastics from nauplii to cyprids, nauplii mortality, development time, growth and metamorphosis rate were not significantly different from the control which didn't receive any microplastics. Moreover, feeding ability of nauplii were barely affected at the highest concentration (1000 beads  $\text{ml}^{-1}$ ). Nauplii appeared to prefer feed on microplastics, when microalgae and microplastics were presented at the same amount. After ingestion, microplastics was egested together with digested algal materials within two hours. Fecal pellets contained microplastics with digested algal materials sank to the bottom rather than float in water column. As a result, ingestion of microplastics by zooplanktons can transform microplastics to the benthic environment.

Keywords: microplastics, barnacle larvae, ingestion, mortality

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# Physiological responses of *Crassostrea virginica* larvae exposed to microplastics

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Microplastic contamination is ubiquitous in the aquatic environment with projections showing an increase in microplastic load worldwide. A growing body of evidence shows that microplastic

ingestion causes harmful effects in many marine organisms including fish, aquatic mammals, turtles, zooplankton, and adult bivalves. The larval stage of the Eastern oyster (*Crassostrea virginica*) is the most vulnerable stage, compared to adults of this species, and therefore is likely the most susceptible to pollutants. However, experiments looking at the effects of microplastic ingestion are lacking for this life stage. This study investigates the physiological response of *C. virginica* larvae during exposure to microplastics. Two polymers commonly found in the coastal environment were chosen for the microplastic exposure solutions: polyethylene terephthalate (PET, polyester) fibers (14 x 42  $\mu\text{m}$ ) and polystyrene microspheres (50/50 mix of 2 and 6  $\mu\text{m}$  diameter spheres). Larvae were exposed to one type of microplastic solution (fibers or spheres) at 100 microplastics  $\text{mL}^{-1}$  at three different ages (7, 9 and 10 days old) for 4, 2, and 1 days respectively. Physiological rates (respiration, algal ingestion, carbon assimilation, and growth) were measured on days 1, 2 and 4, before and after exposure to microplastic solutions. Results will be presented and discussed based on the effects from microplastic type and shape and the duration of exposure.

Keywords: *Crassostrea virginica*, microfiber, polyethylene terephthalate, microsphere, polystyrene

\*Speaker

## Assessing effects of microplastics' ingestion in fish: from innate immune responses and intestinal barrier function to hepatic stress

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The accumulation of plastics in digestive tracts of aquatic biota has been extensively documented and ingestion has been proposed as a prominent exposure route for plastic debris for a variety of aquatic animals, including fish. While the majority of studies investigating effects of microplastics (MPs) have focused on lower micro-size fractions, impacts of MPs from upper micro-size range (> 100  $\mu\text{m}$ ) at which particles are detected in environmental samples or reported ingested by aquatic animals, remain rarely studied. The present study aimed at investigating physiological effects associated with polystyrene (PS) MPs (100-400  $\mu\text{m}$ ) exposure and particles' ability to act as vectors for environmental pollutants thereby inducing chemical toxicity. Herein, we explored whether ingestion of MPs can cause inflammation, intestinal barrier dysfunction or hepatic stress in fish. For 28 days, rainbow trout were fed diets containing untreated PS particles or particles deployed in situ in sewage and harbor effluents. Functional and structural integrity of

intestinal tissues was investigated with Ussing chamber technique, where transport of radioactively-labeled molecules and electrochemical parameters were assessed. Analysis of expression levels of immune-related genes and tight junction proteins was performed to examine if PS MPs and co-contaminants induced inflammation in intestinal tissue. Lysozyme stability and complement system in blood plasma were measured as proxy for systemic inflammation. Markers specific for exposure to common contaminants such as PAHs, endocrine disrupting compounds and metals were used to investigate hepatic stress. We could not show that PS MPs induced inflammation or acted as physical and/or chemical hazards upon ingestion, exerting no measurable effects on intestinal permeability, active transport or electrochemistry in intestinal tissue, or inducing adverse hepatic stress in fish.

Keywords: Oxidative stress, polystyrene, vector effects, biomarkers

\*Speaker

## Do chemicals transfer to or from microplastics in the gut?

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Microplastics acting as a vector for bioaccumulation of hydrophobic organic chemicals (HOCs) is a long standing hypothesis within the research on plastic debris. Previous studies have demonstrated that microplastics contributed to 1-2 times increase or decrease in bioaccumulation of HOCs. These studies were mostly in-vivo with exposure scenarios favouring uptake of chemicals from microplastic, whereas in the environment, chemical uptake by plastic might also occur. Furthermore, the exact transfer kinetics of the HOCs between the ingested microplastics and organisms in the gut cannot be quantified in such tests. Simulating gastrointestinal conditions with artificial gut fluids is an experimental technique to assess bioavailability of chemicals in an organism and can better test the sorption mechanisms. The uptake of chemicals by microplastics from contaminated food has not been studied before. Here, we demonstrate different exposure scenarios of microplastics in artificial gut fluids and transfer of a range of PCBs to/from LDPE and PVC. The data was modelled with a biphasic multi-compartment dynamic model, accounting for reversible transfer of chemicals between microplastics, water, gut fluid and natural food components under different relevant uptake scenarios. Desorption half-lives range from 1.5-3.7 h for LDPE and

0.7-23.9 h for PVC. We demonstrate how organisms with different gut residence times will be affected differently by the ingestion of microplastic. In nature, the levels of chemical mixtures in the biota and microplastics may not be at equilibrium and direction of transfer depends on the fugacity in different compartments.

Keywords: Bioaccumulation, sorption, desorption, kinetics

\*Speaker

## Effects of polystyrene microplastics in different life stages of brown trout (*Salmo trutta f. fario*)

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The widespread use of plastic in our daily life has led to a worldwide pollution of aquatic and terrestrial environments with macro- and microplastics. Nevertheless, knowledge on effects of microplastics in freshwater organisms is still scarce. The aim of our study is to investigate effects of polystyrene microplastics (cryogenically milled granules, fractionated to *Salmo trutta f. fario*). For that purpose, we conducted two fish early life stage tests (FELST) according to OECD 212. We investigated potential effects of polystyrene particles either alone (100; 10 000; 100 000; 1 000 000 particles/L) or in combination with the antidepressant amitriptyline. The investigated endpoints were heart rate, hatching success, mortality and behavior. After consumption of yolk sac by the fish larvae, we additionally investigated the oxidative stress level by means of the ferrous oxidative xylenol orange assay (FOX-assay) and measured the superoxide dismutase activity. In a second experiment, we examined effects of polystyrene particles (10 000 particles/L) alone and in combination with the pesticide methiocarb in juvenile brown trout. In this experiment, mortality rate, level of oxidative stress, proteotoxicity (induction of Hsp70), and inhibition of acetylcholinesterase were under investigation. Furthermore, we examined histopathological effects in gills, liver and gut of the trout. Except for a discussable effect on behavior in the experiment with amitriptyline (Fig. 1) our experiments did not show any negative impact of microplastics on brown trout up to now. Further analyses are in progress. The present study is part of the joint research project "MiWa" (microplastics in freshwater systems) funded by the German Federal Ministry of Education and Research (support code: 02WRS1378).

Keywords: Polystyrene microplastics, Freshwater, Modulation of organic pollutants, Fish

\*Speaker

## Assessing the impact of environmental microplastics in fish using cell line, embryos, larvae and juvenile

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Microplastics (MPs) are tiny plastic fragments, with size < 5mm, widely present in aquatic environment. MPs can be ingested by many organisms, including fish, and can cause chronic physical and/or toxicological effects. Microplastics are the support for many pollutants present in aquatic environment, especially hydrophobic substances. Although numerous studies have documented the microplastic particles uptake and retention in various organisms, impacts on aquatic organisms are little studied so far. In this aims, different approaches will be presented to evaluate the potential toxic effects in fish. Rainbow trout liver cell line RTLW-1 was used to evaluate the toxic effects of organic extracts of environmental samples of microplastics with different assays (MTT, EROD, comet assay). In addition, *in vivo* studies were conducted at various stages of Japanese medaka exposed to microplastic organic extracts, directly to microplastic particles or via trophic contamination. Different biomarkers were evaluated including mortality, biometry, malformations, EROD activity, DNA damage and behavior. Environmental samples were collected during the Race for Water Odyssey in 2015 on beaches of marine islands located in microplastics accumulation area including Hawaii, Bermuda, Guam, Azores, Easter island. For environmental microplastics, different patterns of toxicity were observed in cell line, probably linked with environmental conditions or polymer compositions. Dose-dependent effects were observed on embryos exposed to the organic extract of Hawaii sample on EROD activity induction, biometry and fish reaction to light stimulation. Various toxicity endpoints were induced on larvae after trophic exposure to environmental MPs, which either indicate chemical toxicity of plastic additives and/or sorbed pollutants or physical effects of MPs. Reduced impacts were shown on medaka juveniles

following trophic exposure to MPs probably due to a lower sensitivity compared to larvae. Microplastics from the field were shown to be toxic both on fish cell line and on fish embryos and larvae using different exposure routes.

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Keywords: Fish cell line, Fish embryo assay, Water and Trophic exposures, Microplastics, Sorbed pollutants, Toxicity endpoints

## A mid-scale temporal and spatial analysis of microplastic ingestion in the deep-water red shrimp *Aristeus antennatus*

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Microplastic (MP) ingestion has been confirmed in a wide variety of marine organisms, yet percentage of occurrence greatly varies among species and areas. The present study aims to evaluate mid-scale spatial (230km) and temporal (2007 to 2018) variations in the ingestion of MPs in the deep-sea red shrimp (*Aristeus antennatus*) from the Northwestern Mediterranean Sea. Three different localities (in the north, central and south areas of the Catalan coast), apparently under different levels of anthropogenic pressure were sampled for shrimps in 2018. Moreover, a particular area close to Barcelona city (at about 30 km off the coastline and 800m depth) was sampled on several occasions during the period 2007-2018. Biological characteristics of the shrimps were recorded in addition to the presence of MPs in order to assess the potential effect of MPs on shrimp's condition. Microplastics, mostly fibers with lengths ranging between 0.2 and 30mm, were found in both stomach and intestine contents. In some cases, these fibers were not isolated but tangled up into balls inside the stomach and their diameters reached values around 0.2cm. Overall, percentage of MP occurrence in shrimps was high, with more than 75% of the individuals with MPs in each sampling point. The close relationship of *A. antennatus* with substrate, where shrimps actively prey on benthos, might favor a great exposure to MPs that would be ingested during its feeding activity. Notwithstanding, clear effects of MP overall occurrence on shrimp's condition were not observed. Differences between locations and periods were found, especially concerning ball occurrence, with higher values in southern sampling points (rather than northern ones) and past

periods (2008), in the vicinities of Barcelona. These and other findings that will be discussed point out the potential use of *A. antennatus* as bioindicator species for monitoring MP levels in the Mediterranean Sea.

Keywords: microplastic ingestion, fibers, *Aristeus antennatus*, Mediterranean Sea, Deep Sea, spatial and temporal variation

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## **November 23rd – Panel 22.2: 14h30' – 16h30' (AGH), Panel chaired by Carolin Völker.**

### **Riverine macroplastics in Jakarta**

Michelle Loozen \*† 1, Kees Van Oeveren ‡ 1, Tim Van Emmerik § 2

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Plastics scattered across the land are transported to the ocean by rivers. They are even the main supply of marine plastics. Because of an increased interest in identifying the main polluting countries in the world, several models have been made to estimate the outflow of riverine plastics. However, due to a lack of data the obtained estimations are unvalidated. Recently, The Ocean Cleanup developed a monitoring method to assess quantity, location (longitudinal, cross-sectional and in the water column) and dynamics of riverine plastics. With this knowledge the riverine plastic outflow volumes could be estimated on a daily, monthly and yearly basis which on its turn enables validation of model results.

In May 2018 this monitoring method is applied at 5 locations in the neighborhood of the city of Jakarta. For all locations the plastic density (particles/m<sup>2</sup>) in time and space has been determined and the ratio between organics and plastics identified. The composition of the riverine plastic has been identified and the characteristics per plastic type investigated. In short summary of the findings; the plastic particle flux (particles/min) appears to be related to the flow velocity (m/s) and the flow velocity appears to be related to the submergence of plastics. Furthermore, a difference between the composition of surface plastics and water column plastics has been found. The results of this fieldwork contribute to the understanding of the dynamics and composition of plastics and will eventually enable validation of the above mentioned global model.

Keywords: River, macroplastics, Jakarta, monitoring method, water column distribution, spatial distribution

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# Microplastic prevalence in benthic macroinvertebrates: preliminary evidence of microplastic particles among primary consumers in a lotic environment

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Microplastic (MP) polymers (1  $\mu\text{m}$  – 5 mm) are ubiquitous within aquatic environments. Due to their small size there is a risk that they may infiltrate and transfer within aquatic food webs. Although there is experimental evidence to suggest that freshwater biota can readily ingest MPs, particularly at lower trophic levels, the lack of field data means that our knowledge of MP prevalence among biota in the freshwater environment is limited. MP exposure is primarily determined by the feeding strategy of the organism, the characteristics of the polymer type and where within the environment they occur. As such, MP exposure is assumed to be especially relevant for benthic macroinvertebrates, which interact and feed close to the benthos where MPs are likely to accumulate. The aim of this research is to quantify and characterise the MP burden imposed on benthic macroinvertebrate communities within a lotic system in Ireland, as a means of investigating potential pathways of MP assimilation within the freshwater food web. Ten, one-minute kick samples were obtained from three reference sites and three high exposure sites along the River Slaney catchment. Taxa were identified to family level for biological assessment of water quality, and subjected to a 48 hour digestion in a 30 % HO (Hydrogen Peroxide) v/v solution to isolate MPs from invertebrate integument. Preliminary results indicate MP prevalence to be highest among the functional feeding group (FFG) shredders, which typically feed on coarse particulate organic matter within the benthos, with fibres the most frequent polymer found followed by fragments. The high prevalence of MPs among shredders could provide evidence to suggest that this FFG is at a greater risk of ingesting MPs and transferring them to higher trophic levels.

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Keywords: freshwater ecology, food webs, polymers, water quality, water pollution

# (Micro)plastics in Sediment and Water Samples of the Rivers Elbe and Lahn, Germany

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Plastic pollution in the aquatic environment has gained worldwide attention in the last years. Meanwhile, intensive research activities have been initiated in these environments; however, the effects and consequences of the plastic pollution are not fully known and have to be better understood. In the frame of the project "Microplastics in German waterways", samples from 11 sites from the river Elbe between Dessau and Vogelsang as well as four sites of the river Lahn upstream and downstream of the effluent of a waste water treatment plant were taken in order to study the plastic pollution in water and sediment and to show the differences of sampling methods.

The sediment samples were taken with a Van-Veen-grabber, the water samples from the Elbe with an Apstein plankton net (mesh size 150  $\mu\text{m}$ ) and from the Lahn with a flow centrifuge and a plankton net (5  $\mu\text{m}$ ). The sediment samples were presorted with wet sieving, organic digestion and density separation, filtered on aluminium oxide filters and visually analysed. For the water samples, the organic matter was digested using a reagent composed of equal volumes of 10 M KOH and 30 % H<sub>2</sub>O<sub>2</sub>, then, the (micro)plastic particles were isolated from remaining matrix by density floatation using 1.6 g/mL potassium formate solution and pressure filtration. Analysis was done by visual inspection, selected particles measured with pyrolysis GCMS and Fourier-transform infrared spectroscopy.

The results of the sediments of the Elbe point to a (micro)plastic concentration depending on the sampling site (esp. polystyrene and polypropylene particles) and to a decrease in the flow direction whereas the water samples only show a site specific (micro)plastic concentration. The Lahn samples clearly reveal a higher concentration of particles in the samples downstream the effluent of the waste water treatment plant.

\*Speaker

Keywords: (micro)plastics, river, freshwater, Germany

# Spatio-temporal distribution of microplastics in water and sediments of a southern european river (Antuã a river, Portugal)

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The accumulation of plastics in aquatic systems, especially microplastics (MPs, particles with < 5 mm), is of particular apprehension since they can reach high densities, derive from a variety of sources and can interact with biotic and abiotic environment. The occurrence of MPs in freshwater systems is less understood than in marine environment. Hence, this study aims to fill this knowledge gap providing new insights into MPs contamination in Antuã a river in water and sediment samples collected in March and October of 2016 in several stretches of the river. The abundance of MPs reached  $14.3 \pm 18.3$  mg m<sup>-3</sup> or  $306.4 \pm 472.1$  items m<sup>-3</sup> in water samples and  $35.8 \pm 25.7$  mg kg<sup>-1</sup> or  $318.9 \pm 246$  items kg<sup>-1</sup> in sediments. Spatial and temporal distributions show different pattern according to seasonal conditions, proximity to urban areas and flow velocity. The water and sediment samples with the greatest abundances were São João da Madeira (SJM) and Aguincheira, respectively. In water compartment, the highest abundance of MPs was observed in October, while in sediments an opposite pattern was observed. Fourier transform infrared spectroscopy (FTIR) plastics' analysis underline polyethylene (PE) and polypropylene (PP) polymers as the most common types covering more than 50% of all polymer types identified. Foams and fibers were the most abundant type in SJM, while fibers and fragments were the most abundant in Aguincheira and Estarreja in water and sediment samples, respectively. Since Portugal is the 12th country in Europe with the highest plastics demand (1 mt) and 10%-50% of plastic go to landfills, it is urgent to monitor its freshwater systems. This study emphasizes the importance of rivers as potential carriage systems of MPs within environment. Further studies should be performed to identify point sources in order to mitigate the MP contamination in aquatic systems.

Keywords: Contamination, Microplastics, Water, Sediment, Antuã a River

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# Microplastics in two contrasted rivers discharging into the Northwestern Mediterranean Sea

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Freshwater environments are exposed to microplastic (MP) pollution due to the use and discard of plastics on adjacent lands. Rivers are suspected to be a major transport pathway of MPs from terrestrial to marine ecosystems. Monitoring MP concentrations within rivers is therefore critical to assess hazards on freshwater ecosystems, but also to estimate MP fluxes from watersheds to coastal waters. However, only a few studies have investigated MP concentrations in rivers. To fill this gap, MPs (size < 5 mm) were collected on several occasions during a complete yearly cycle using a manta trawl (> 300 µm) at the surface of two contrasted rivers: the Rhône River, the largest source of freshwater and sediments into the Mediterranean Sea and a typical small Mediterranean coastal river, the Têt River. MP concentrations varied by two orders of magnitude for both rivers: from 0.01 to 0.66 mg.m<sup>-3</sup> (mean = 0.13 ± 0.17 mg.m<sup>-3</sup>) for the Rhône River and from 0.02 to 3.41 mg.m<sup>-3</sup> (mean = 0.87 ± 0.86 mg.m<sup>-3</sup>) for the Têt River, which was significantly more polluted (Wilcoxon test; p.value < 0.001). Fibers were the most abundant shape (95-85%), followed by fragments (10-2%). Foams, films and beads were less represented (< 2%). FTIR analysis indicates that fibers were mainly made of polyester (PES), fragments and films were mostly polyethylene (PE) and polypropylene (PP), while foams were essentially made of polystyrene (PS). Replicate sampling was performed on the Têt River that showed that the short-term temporal variability (hours) was as important as the seasonal variability (months). No clear trends were found for both rivers between MPs concentration and external factors such as local precipitations, water flow rates and total suspended matters, indicating complex or chaotic patterns of MP distribution within river waters and subsequent transport.

Keywords: microplastics, anthropogenic litter, rivers.

\*Speaker

## Microplastics in the Italian lakes: main results of two monitoring years

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Despite the large amount of literature describing microplastics occurrence and accumulation in marine environments, yet very little data are available for freshwater systems, and, particularly, little is known as regards the situation in Italian lakes. Starting from 2016 Legambiente and ENEA are carrying out a joint research on microplastics (1-5 mm) presence on the main Italian lakes. The greater mean concentration for Subalpine lakes was recorded in Lake Iseo, 40000 particles/km<sup>2</sup>, 39000 particles/ km<sup>2</sup> in Lake Maggiore and 10000 particles/km<sup>2</sup> in the Garda Lake. The highest particles concentration was found in transect located in the part of the lacustrine basins directly affected by the presence of water inflow of rivers affected by sewage pollution. In 2017 the study focussed on fluvial input and output in the lacual basins in order to highlight the influence of the wastewater treatment plant and appears that the difference detected between the samples taken downstream and upstream of the wastewater treatment plant can reach up to 80% of particles/m<sup>3</sup>. There is an undeniable relationship between municipal discharges, sewage, urban runoff and storm water from rivers-lakes system to sea. Regarding the regions of central Italy Bracciano and Trasimeno Lakes were monitored. In Bracciano Lake the average density was 117000 microparticles/km<sup>2</sup>, and a peak of 554000 microparticles/km<sup>2</sup> was recorded. The environmental situation of Lake Bracciano in 2017 was particularly critical because of the high evaporation, the deficit of rainfall and the water captions, as the lake is used as a drinking water reservoir for the city of Rome and the Vatican State. Considering the Trasimeno Lake, one of the largest Italian lake but also one of the shallower (5 meters, on average), the mean concentration recorded in 2016 was 8974 microparticles/km<sup>2</sup> in line with the mean of 7914 microparticles/km<sup>2</sup> of 2017 monitoring.

## Microplastics monitoring in water bodies of the North-West of Russia

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The problem of microplastic pollution can become a new and underestimated threat for such unique and fragile aquatic ecosystems like the brackish Gulf of Finland (the Baltic Sea) and Ladoga Lake - the largest freshwater lake in Europe. In 2017 year three Russian Institutes started to survey the water bodies of Saint-Petersburg and the Leningrad oblast to determine the levels of microplastic pollution. Particular attention was drawn to Ladoga Lake and the Neva river as the objects of strategic importance for the water supply of metropolitan area of Saint- Petersburg, as well as the Neva estuary in the easternmost tip of the Gulf of Finland - highly polluted and eutrophied water body. Ladoga Lake and Neva estuary being part of one aquatic system are very different in terms of morphology, hydrodynamics and hydrochemical regime. Thus, several microplastic sampling methods have been tested in order to elaborate a unified method for this region. Microplastic particles concentration in water column was determined by the light microscope based on the methods of Marine & Environmental Research Institute, USA. The first results from 2017 year show that microplastic particles were detected in all samples from the water bodies of the Neva catchment area. On average several particles per liter were registered in every sample. Data on microplastics concentrations and composition in natural water bodies represent some features of plastic particles transformation and transportation in water column and into the bottom sediments. First beach monitoring surveys in the Neva Bay (2018) showed high accumulation of macro- and meso-litter particles in beach sand, however, not so many particles 2-5 mm was found. Samples of bottom sediments in this area were studied by means of light microscope and IR-spectrometry methods. Preliminary results show the possibly importance of bottom sediments as an accumulation hotspot for micro-particles.

Keywords: microplastics in water and bottom sediments, the Neva river, Gulf of Finland, Ladoga Lake

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# Posters



The students from the "Red Canaria de Centros Educativos GLOBE" will be in front of their posters and exchanging questions with other participants on Wednesday 21st during the Posters session, 13h-14h30'.

## Microplastics distribution in surface waters in the Western Mediterranean Sea

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Is possible to find microplastics (< 5 mm) even in the most remote areas on the planet, this includes the surface waters in the Mediterranean Sea. In the open ocean, this kind of pollution is introduced from terrestrial sources, including inadequately disposed plastic, effluents of waste water treatment plants or via river plumes. Plastic materials tend to accumulate in the anticyclonic gyres and convergence zones in the open ocean. In this study, during RADMED 0216 cruise, surface samples were collected in 5 orthogonal sections to the coast along bathymetric gradient with a manta trawl net (333 µm mesh), in 23 sampling stations (from Catalonia to Alborán). We analyzed the abundance, the chemical characterization of microplastics and the distribution of plastic particles. Microplastics were isolated using the visual identification and counted, and then a representative 10 % were selected and classified by shape, colour and size using also a stereomicroscope NexiusZoom (Euromex). Furthermore, this 10 % were identified by ATR-FTIR spectroscopy.

In this survey, abundances are in the order of 0.5 particles by square meter of sea surface. The highest concentrations, up to 515 particles m<sup>-3</sup>, were detected along the Ebro river mouth sampling transects.

Density of microplastics (except microfibers) in Spanish Western Mediterranean during RADMED 0216 survey. To better understand the accumulation zones of microplastics we will analyze the relationship with other variables as the distance to the coastline and to convergence zones among others.

Keywords: Western Mediterranean, microplastics

\*Speaker

## Microplastics in sea water of Cienfuegos bay, Cuba

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Microplastics are found principally in closed bays with industrial activity. The Cienfuegos Bay, is a closed bay typical where has cuantificated different pollutants but no exist report about the presence of microplastics in these ecosystems. In these work we set out to make a studied in surface sea water for evaluate the presence and density of microplastic in the dry and wet season in the Cienfuegos Bay. They were observed microplastic in sea water in all station evaluated. The average of the values found in the dry season was 49 562.5 microplastic/m<sup>3</sup> in a range between 3000 and 581 500 microplastic /m<sup>3</sup>, reporting the high concentration in the station 12, place very near of Cienfuegos city. In the wet season found an average the 5093.75 microplastic /m<sup>3</sup> in the range between 0.0 and 10 500 microplastic/m<sup>3</sup>, showing the high concentration in the station 16, located at the mouth of the Laguna Guanaroaca.

Keywords: Microplastic, Pollution, Cienfuegos Bay, Sea water

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## Osteotoxic effects of polycyclic aromatic hydrocarbons adsorbed to microplastics

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Microplastics present in aquatic ecosystems and in the food web represent a major concern for the ocean and human health. Adverse effects such as growth inhibition, reproductive dysfunction, feeding disorders and mortality have been described in different trophic levels. In addition, recent reports showed that contaminants such as polycyclic aromatic hydrocarbons (PAH), known to disrupt endocrine system and impair skeletal development and bone formation/maintenance, have the ability to adsorb at the surface of microplastics, raising additional concerns about the adverse effects produced by the presence of microplastics in the environment. In this work, the osteotoxic

effect of 2 model PAH compounds with the potential to be adsorbed to microplastics – benzo[a]pyrene (BaP) and 3-methylcholanthrene (3-MC) – were assessed in zebrafish (*Danio rerio*) using several in vivo bone-related systems. Fish were waterborne exposed to BaP and 3-MC (dissolved in DMSO), and the effects on the operculum development (larvae), caudal fin and scales regeneration (adult) and levels of skeletal deformities (post-larvae) were assessed through bone specific staining and morphometric analysis. BaP and 3-MC reduced the growth of the opercular bone in a dose-dependent manner, impaired de novo bone formation in regenerating caudal fin and scales, and increased the number of skeletal deformities, demonstrating the osteotoxic and skeletotoxic potential of PAHs. This work provides new insights into the osteotoxic effects of PAH and essential data to further assess the effects of environmental pollutants with the potential to be adsorbed to microplastics.

Keywords: Polycyclic aromatic hydrocarbons, Osteotoxicity, Zebrafish

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## On the representativeness of bulk water samples versus manta sampling in microplastic analysis

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The harmonization of methodical approaches along the complete process chain beginning with sampling-concepts and ending with the identification respectively characterization of microplastic particles is of major relevance for future investigations. Manta trawling is most commonly applied in water sampling for microplastic analysis, though, the mesh size sets an operational detection limit (mostly at 300 or 333µm). It is widely agreed, that the majority of particles found is of smaller size which gave rise to set up new strategies involving bulk sampling with pump systems. Still there is uncertainty about the minimum volume of water required to meet the important criteria of representativeness. Here, first results regarding the comparability of a novel bulk water sampling system and a manta trawl are presented, exemplarily investigated in the freshwater model catchment of Lake Tollense (Mecklenburg-Western Pomerania, Germany). This catchment is characterized by favorable conditions with respect to the lake's shape and dimensions, its exposure within the landscape, strong gradients concerning human activities and the polarized allocation of its tributaries and discharge that enable the investigation of sources and driving factors for microplastic pollution in freshwater ecosystems and thus, the generation of a budget. Samples were taken along eight manta transects with an additional volume bulk sample in the midpoint of each transect.

Sampling volumes comprised 3000l in total, being taken in four subvolumes (2x500l, 2x1000l). Based on statistical investigations of the sample aliquots the minimum volume of water required will be assessed. On board of the vessel the bulk samples were volume reduced implementing a sieving cascade with various mesh sizes (shown in figure). Above all, the size fractioning prevents especially smaller sieves to clog and thus, enables the sampling of large water volumes.

Keywords: bulk water samples, manta trawl, representativeness, freshwater

\*Speaker

## Microplastics in the municipal wastewater treatment plants at Turkey: Comparison of the influent and secondary effluent concentrations

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Wastewater treatment plants are one of the important pathways via which the microplastics enter the aquatic ecosystem. In this study, we have established the microplastics concentrations of the influent and secondary effluent water of two wastewater treatment plants in Turkey. For this purpose, we have taken samples of the influent and effluent water of Seyhan and Yüreğir wastewater treatment facilities for 6 days in August 2017 and determined their microplastics' content. It was found that the influent of the wastewater treatment had 1 million to 6.5 million particles per day, and the effluent had 220,000 to 1.5 million particles per day. It was determined that the removal rate of microplastics were found between 73% and 79%. In total, eight different types of polymers were detected. The most frequently found polymer type was polyester. The average annual microplastics particles per household were found to be 746 particles/year/household.

Keywords: Wastewater Treatment Plant, Microplastic, Microfiber, Plastic pollution, Turkey

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# Distribution of microplastics along the Scottish coastline using standardized sampling and extraction methods

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Approximately 13 million tons of plastic enters the oceans globally each year. Microplastics are particles less than 5mm and due to their specific characteristics, they have become ubiquitous in the marine environment. Microplastic particles have been found in marine sediments, water and organisms. Current literature indicates that most of the plastic debris originates from the terrestrial environment, and is contributing to the widespread distribution of microplastic along shorelines around the world.

Despite a plethora of studies investigating microplastic abundance, it is difficult to make comparisons due to the variety of protocols and measurement units used across different studies. To answer the growing demand to create a parsimonious methodology, this study has developed and adapted standardised, simplified protocols to sample three different elements (i.e. coastal water, intertidal sediment and mussels) along the Scottish coastline. This research has investigated several locations based on factors including the proximity of populated and heavily commercialised areas, hydrodynamics, and tidal height, to better understand their role in the distribution and abundance of microplastics. A canola oil based extraction technique is used during sample processing to recover plastic from intertidal sediment; while a trypsin enzyme digestion is adapted to extract microplastics from biological fauna.

These protocols are simple to reproduce, efficient, fast and cheap allowing the study of microplastic pollution in a range of samples. Results are expected to highlight a heterogeneous microplastic distribution based on hydrodynamics. Microplastic abundance will be higher in sheltered and heavily populated areas compared to hydrodynamically active and low populated sites for water and sediment. This research will fill a knowledge gap regarding the presence and abundance of microplastics around the coast of Scotland, and advance our understanding of key factors that play a role in determining plastic distribution.

Keywords: microplastics, sampling and extraction technique, sediment, coastal water, mussels

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## Antifouling paint particles in coastal and estuarine ecosystems

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Microplastics (0.1  $\mu\text{m}$ –5 mm) are a widespread and abundant marine contaminant that presents a risk to marine life and healthy ecosystems. Antifouling paint is applied to the hull of ships, boats and other marine structures to prevent biofouling. These paints contain high concentrations of metals, including copper (Cu) and zinc (Zn), which are toxic to marine organisms. Antifouling paint particles (APPs), which are classed as a type of microplastic, are generated during cleaning and maintenance of marine structures in boatyards and marinas. These fragments are often transported from hard-standings and slipways into the marine environment where they may be ingested by deposit- and suspension-feeding organisms. This study investigated the prevalence of APPs and associated metals in estuarine sediments and sediment-dwelling biota. Field sampling was conducted at eight sites across the Plym and Erme estuaries (UK); at each site we undertook randomised sediment sampling (n=5 per site) and ad libitum sampling of the ragworm (*Hediste diversicolor*) and common cockles (*Cerastoderma edule*). Samples were processed to isolate APPs, and metal analysis conducted using XRF, ICP-MS or ICP-OES. Our environmental analyses demonstrate a heterogeneous distribution of APPs, with highest concentrations associated with abandoned boats, where we identified larger paint particles rich in Cu, Zn, tin (Sn) and lead (Pb), and marinas, where we identified smaller paint particles rich in Cu and Zn. We identified a relationship between antifouling metal concentrations in sediment and biota, suggesting ingestion of APPs in the natural environment and accumulation of metals in tissues. Our study suggests APPs are prevalent in estuarine and coastal ecosystems with high boat maintenance activity, where they pose a substantial risk to marine life.

Keywords: Antifouling paint particles, estuarine sediment, *Hediste diversicolor*, *Cerastoderma edule*

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# Who bit it? A field guide to bite marks on plastic pollution

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Ingestion of marine plastics has been documented in numerous invertebrates, fish, seabirds, sea turtles and marine mammals, as well as the negative impacts on these animals. However, little attention has been given to fragmentation and biomechanical degradation of plastics by animal grazing and biting, or its contribution to the formation of secondary microplastics. Here we summarize the information from published literature and our field observations on the evidence of interaction between marine and land organisms and plastic debris. A variety of bite marks, pecking, grazing and scraping marks have been reported on plastic debris, including marks left by microorganisms, invertebrates, and many vertebrates on land and at sea. Fragmentation of plastics by amphipods and polychaete worms in laboratory conditions has also been described. In terrestrial environments, plastic ingestion has been reported in the literature by camels, goats, cows and sheep. Additionally, we observed fragmentation of plastic objects by rodents and dogs, leaving shredded bits behind and distinct bite-marks on the plastic. The fragmentation of plastics into microplastics on land makes recovery more difficult and facilitates easier transport to the marine environment by rain and wind. We provided a photo-documentation of a number of bite marks with a corresponding marine or land organism responsible for leaving the marks, which will serve as the basis for the bite mark identification field guide.

Keywords: bite, marks, grazing, biomechanical degradation, ecological impact

\*Speaker

# Is Phytoplankton dying to tell us its Nanoplastic story?

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As methods for detecting nanoplastics in marine samples increase in accuracy and resolution, a clearer picture of environmental distribution of these particles emerges. But, we are still out of our depth in some key factors of the oceanic nanoplastic story as their fate and environmental impact is neither understood nor fully investigated. Small plastic particles are not conducive to plankton

health, that much is transparent. Among the effects are enhanced production of reactive oxygen species, a reduction of CO<sub>2</sub> uptake and chlorophyll-a pigment levels as well as overall growth rate. But are tiny plastics directly causing this or do they merely cause nutrient limitations resulting in the stressed plankton? How bad are these ubiquitous particles? Do present or future levels present a threat to the sustainability of oceanic plankton communities? How resilient are plankton communities that inhabit the plastic filled gyres to this additional stress in the face of changing water temperatures and rising pH? Will there be enough genetic variability in these oligotrophic ecosystems to sustain a change to an equally productive plankton community or will the stress be too much, and the reorganization of this millennia old biome results in a reduction of its potential to provide essential ecosystem functions such as the contribution to our atmospheric oxygen budget. In order to limit the fishing in the dark and answer those questions, we look at RNA expression and photosystem efficiency under present and future conditions to be able to make more exact predictions about changes in these plankton communities of the open ocean. As plastics are now a part of every marine environment, creating a complete picture of chemical and physical effects of nanoplastic on marine plankton will need to start with the lowest trophic level, phytoplankton.

Keywords: Marine plankton communities, nanoplastics

\*Speaker

## Evaluation of various digestion protocols within microplastic sample processing concerning their efficiency of organic matter destruction and the resistance of different polymers

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The destruction of biological organic material is an essential step of sample preparation within microplastic analyses. Organic residues hampers the separation of polymer particles especially within density separation or polymer identification via staining methods (e.g. with Nile red). Therefore, a concise literature survey on 104 scientific publications has been undertaken to identify the most commonly applied digestion protocols on samples of water, sediments and biota. The selected protocols comprise different solutions, concentrations and reaction temperatures. Within this study we tested acids (nitric acid 20% and 65 %, hydrochloric acid 10% and 37%), bases (sodium hydroxide 10%, 20% and 40%, potassium hydroxide 3.75% and 15%), oxidizing agents



(hydrogen peroxide 30% and 50%, sodium hypochlorite 2-5% and 6-14%), and Fenton's reagent (hydrogen peroxide 30 % and iron(II)sulfate) at three different temperatures (room temperature, 40-50°C and 60-70°C) on their efficiency of organic matter destruction and the resistance of different polymers against the procedures. Tests were applied on organic material (soft tissue - leaves, hard tissue – branches and mussel shells) and 6 polymers (LDPE, HDPE, PP, PA, PS and PET) in two size categories each (1-5mm and 0.3-1mm) and three parallels each. Results show that the application of nitric acid and sodium hypochlorite are most effective (> 80% in the median) for organic matter destruction. Increasing the reaction temperature up to 40-50°C enforced the reaction significantly concerning most protocols while the further enhancement up to 60-70°C did not show a distinct improvement. Polymers are mostly affected through the application of nitric acid and sodium hydroxide (especially PS, PET and PA).

Keywords: sample treatment, digestion protocols, organic matter, polymer resistance

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## First assessment of driving factors for microplastic pollution within the Lake Tollense catchment

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In July 2016 the microplastic pollution in the Lake Tollense catchment in Mecklenburg- Western Pomerania, Germany was assessed implementing Manta-trawling and bulk water sampling via a pump system. Samples were taken within six transects with a Manta trawl of 300 µm mesh size. Manta sampling was repeated to investigate potential short-term temporal variation. Two additional bulk samples (100l each) at the midpoint of each Manta transect in two depths were taken and subdivided into different size categories via a sieving cascade down to a particle size of 63 µm. Furthermore, three tributaries and the discharge of Lake Tollense were sampled, with a sampling volume of 100l at each location in order to evaluate in- and outputs of microplastic particles via the receiving streams. Additionally, land use patterns within the sub-catchments, water temperature and conductivity at the sampling sites as well as stream discharges were recorded. In the laboratory, samples were treated with hydrogen peroxide (30%) and sodium hypochlorite (6-14%) to digest biologic organic matter. Subsequently, filters were stained with Nile Red solution (1mg/ml in chloroform) and examined under a fluorescence microscope (AxioLab A.1, Zeiss). Additionally, microplastic fragments and fibres were measured to evaluate their actual size distribution as it has been reported before that manta meshes might retain particles smaller than their defined opening. A

small subsample was analyzed via Raman-spectroscopy to determine polymer types (DXR2xi Raman Imaging Microscope, Thermo Fisher Scientific). Microplastic particles were present in all samples. First results indicate a considerable variability of MP concentration due to anthropogenic activity within the catchment. Moreover, higher concentrations were present at the surface compared to the deeper water layer and MP abundance increases with decreasing particle size.

Keywords: microplastics, freshwater, manta trawl, bulk sampling, Nile Red, Raman spectroscopy

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## Exploring the impact of microplastic on the Humboldt large marine ecosystem

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Microplastic debris is a prolific environmental contaminant, posing a risk to the health of marine biota, ecosystems and potentially food security. In October 2018, our research team hosted an international training workshop for early career researchers, exploring the transport pathways, uptake mechanisms and prevalence of microplastics in natural ecosystems. A focal point of the workshop was the Humboldt Large Marine Ecosystem (HLME); this biodiverse, highly-productive ecosystem hosts species endemic to the region, and sustains the biggest single species (anchovy) fishery in the world. Owing to its proximity to urbanised coastlines and as a region of upwelling, the HLME is at high risk of microplastic pollution. During the workshop, we investigated the threat microplastic debris poses to anchovy stocks in the HLME. Biotic, water and sediment samples collected throughout the HLME in June-September 2018 were processed using established techniques; isolated microplastics were subsequently characterised, quantified and polymer composition determined using ATR-FT-IR. Our results demonstrate the capacity for anchovy to consume microplastics. We elucidate how microplastic exposure has the capacity to negatively affect the health of anchovy populations, and explore the repercussions for the local economy and welfare of coastal populations in this region. Given the extent to which anchovy are processed into fishmeal and fish oil for use in the global aquaculture industry, we further explore risks posed to sustainable aquaculture, food security and human health.

Keywords: Marine Ecosystems, Human Health, Microplastic, Aquaculture, Food Security

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## Can oysters be used as “passive samplers” for microplastics?

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Microplastics are a pollutant of emerging concern that have caught the attention of the scientific community. With the rapid increase in plastic production and spread, plastic debris are accumulating in the aquatic environment where they fragment into smaller pieces. A range of organisms, especially marine invertebrates, are vulnerable to microplastic exposure, with seafood consumption a route for human exposure, which is an important component of our diet. The aim of this study is to assess the inherent risk of exposure to microplastics through seafood consumption, namely oysters. Rock oysters (*Saccostrea glomerata*) were obtained alive from a local farm (QLD) and a sub-sample of these was set aside to assess background concentration of potential microplastics. The remaining oysters were distributed in stainless steel mesh cages and deployed in two different locations in the Brisbane River - a very urbanised area with high levels of boat traffic and wastewater released upstream. The oysters were left there for 14 days and taken to the lab for further analysis. A range of techniques will be applied to assess the type and concentration of microplastics and additives: visual observation using an optical microscope, fourier-transform infrared spectroscopy (FT-IR) and pyrolysis-gas chromatography coupled to mass spectrometry (Pyr GC-MS). A database for both FT-IR and Pyr GC-MS will be created by measuring not only the most common polymer standards (PET, PC, PMMA, PS, PP, PA66 (nylon), LD/HD PE), but also consumer products commonly found in the environment (fishing nets, tyre, rubber, straws, polymer composite paints, rope, cigarette filters, among others). This provides a “real plastic” library to match with the microplastics found in our samples and develop new techniques to analyse microplastics in marine biota.

Keywords: Oyster, marine plastic debris, seafood, FTIR, Pyr GCMS

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# Macro- and microplastics in sediments from Lake Tollense, Germany

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Freshwater systems need to be considered in detail within the microplastic research since they are the pathways for plastics to the oceans. Especially, limnic basins may act as a (temporary) sink for microplastics within these pathways. This study analyzes exemplarily the abundance and distribution of macro- and microplastics at the Lake Tollense in Mecklenburg-Vorpommern, Germany. The aim is to identify sources for macro- and microplastics and the influences on their distribution in a limnic environment. Therefore, semi-annually, a macrolitter monitoring is performed and sediment samples are taken in several parallels and from different heights at four bank border segments around the lake. The sandy beaches differ concerning their exposition and their anthropogenic influence which might give important insights on probable sources of macro- and microplastic pollution. Macrolitter is predominately composed of plastic (71%) and items are irregularly distributed within the beach segments. A seasonal variability is evident, with a higher macrolitter abundance in September compared to the monitoring in March. Furthermore, anthropogenic influences seem to play an important role for the macrolitter distribution. For microplastic extraction from sediment a first step of digestion of organic matter is applied. Hydrogen peroxide as well as sodium hypochlorite are deployed for this purpose. The final separation process is done via elutriation. Microplastics are identified using the Nile Red staining method and partly via Raman spectroscopy. Microplastics were identifiable at all beaches. First results concerning the difference between the microplastic abundance at different beaches will be presented with regard to anthropogenic and wind as well as lake current influences. Furthermore, it will be analyzed whether the spatial distribution of microplastics show specific accumulation patterns e.g. at the high-tide line of the lake's bank borders.

Keywords: microplastics, macrolitter, lake, sediments, distribution, sources

\*Speaker

# Correlations between the small scaled microplastic pollution and grain size distribution of beach sediments on the Isle of Rügen, Baltic Sea, Germany

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The contamination of the marine environment with microplastics is of growing interest for a few decades now. Marine beach sediments act as an accumulation zone for microplastics and represent a transition zone between the land and the ocean. This study analyzes the abundance of microplastics in beach sediments on the Isle of Rügen, Baltic Sea, Germany. Furthermore it establishes a connection between the microplastic pollution of sediments and the grain size distribution as well as other pedologic parameters (pH, conductivity, C/N ratio, water content, organic matter, bulk density). In addition to the abundance of microplastics this study investigates the small scaled spatial distribution of microplastics across the beach from the water line to the backward dunes. In total, 81 sediment samples were taken in June 2017 at three beaches on the Isle of Rügen. These beaches are characterized by different expositions and anthropogenic influences due to their location and accessibility. Samples were taken at different beach levels to identify the spatial distribution of microplastics across the beach. For the microplastic analysis the essential treatment step for organic matter destruction was performed using a combination of hydrogen peroxide (30%) and sodium hypochlorite (6-14%). The following separation of microplastics from sediment was conducted via elutriation. For the final identification of microplastics samples were stained with Nile Red and digital photos were taken under UV light for the visual count of microplastics. Microplastics were found at all investigated beaches. First results of this study show correlations between the pollution of marine beach sediments and their grain size distribution and identify small scaled spatial accumulation zones of microplastics across the beach. Further relationships between the microplastic concentration and pedologic parameters will be analyzed and presented.

Keywords: microplastics, sediments, grain size distribution, small scaled distribution, elutriation, Nile Red

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# The impact of polystyrene microplastic on physiological activities of *Calanus helgolandicus* in the Marmara Sea

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Microplastics are widely distributed in the marine environment, consumed by marine organisms and can affect their vital functions. Our study is the first evidence of the negative effect of microplastics microplasticity on the energy metabolism of the copepod *Calanus helgolandicus* in the Marmara Sea. Our experimental design incorporated 72 h feeding assays using 6, 12 and 26 µm sized polystyrene beads either fed separately or incorporated with diatom algae. The rate of microplastic bead consumption was determined from the number of fecal pellets containing beads and numbers of beads within the pellets. Energy metabolism levels of *C. helgolandicus* were estimated from respiration rates of active and anaesthetized individuals (total and basal metabolism, respectively). It was found that after the addition of microplastic beads to the water containing natural diatom algae, *C. helgolandicus* began to actively consume beads. The proportion of the smallest-sized 6 µm microbead particles in fecal pellets was significantly greater (1.3 fold) than that in water, indicating the ability of the *C. helgolandicus* to select smaller sized beads.

The respiration rates of active females, feeding both on algae and microplastic, on the third day of the experiment, were found to be significantly lower (1.5 times) than in females feeding solely on algae, whereas levels of basal metabolism displayed no significant changes. In females exposed only to microplastics, significantly lower levels were observed in both total and basal metabolism, especially in individuals consuming the largest (26 µm) microbead particles. Multiple effects as a result of microplastic ingestion are apparent on the energy metabolism such as fasting, bead fouling, compensation for lack of food, behavioral responses to starvation and different particle sizes, traumatic effects of beads on the parenchyma of the intestine as well as the loss of body material during the formation of the fecal pellet shell.

Keywords: microplastics, marmara sea, *Calanus helgolandicus*

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# Biomacromolecules and bioplastics from fish waste for high added value applications

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More than 1.3 million tonnes of Marine rest raw material (MRRM) are generated in Europe each year. Some countries, such as Norway and Denmark, have traditionally for animal feed. It will therefore be a challenge for the industry to develop methods to turn fish viscera and skin, currently considered as undesirable raw materials for hydrolysis and human consumption, into profitable products. One of the main objectives of the DAFIA project is to explore the conversion routes of marine rest raw-materials (MRRM) from the fish processing industries, to obtain high added value products, i.e. flame retardants, edible/barrier coatings and chemical building blocks (diamine) to produce polyamides for a wide range industrial applications.

Gelatine, nucleic acids, proteins and polypeptides and pentanediamine are the main products obtained from MRRM with very interesting and innovative uses applied in this project.

From the high added value products extracted from the MRRM, new bio-polyamides flame retardant compounds suitable for automotive industry, fish gelatine-based active and edible and barrier coatings for application on food and packaging have been developed.

Keywords: marine rest raw materials, bioplastics, gelatine, proteins, flame retardants, packaging, fish, coatings

\*Speaker

# Spatial analysis and prediction of microplastics concentrations using geostatistics and optimization

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The deleterious effect and generalized impact of microplastics in marine ecosystems is of rising concern; however, it still remains poorly assessed and characterized. As a result of plastics overconsumption and inadequate disposal processes, nano- and microplastics have recently been shown to be inadvertently ingested along trophic webs up to top predators. According to Plastox JPI

project, almost 73% of 233 fishes analysed have shown signs of microplastics in their stomach. However, despite the commitment of the scientific community and political entities to mitigate this societal problem, the environmental fate, as well as bioaccumulation and quantification of its extent, still remain underdeveloped. The vast areas affected and the heterogeneity expected for microplastics distribution and abundance are some of the reasons that make its characterization challenging. Large-scale spatial distribution techniques, like geostatistics, may contribute effectively to improve the range of methodologies and their efficiency, due to their proneness to deal with the identification and quantification of microplastic particles in the marine environment. Along with the definition of standardized sampling and recording procedures, these spatial mapping techniques need to be harmonized and validated. Besides the already proven applications of geostatistics in the spatial characterization of the heterogeneities of large-scale variables, only recently the technique started to be applied to maritime variables, namely parameters related to water quality, fish abundance and marine species classification.

This work is focussed on the application of geostatistical techniques to produce the spatial mapping and allow the interpretation of the distribution and abundance of plastic debris using information collected in the North Pacific Ocean. The main variables analysed are concentration rates and particle sizes. High concentration spots were identified after carrying out the analysis. Recommendations towards the locations for additional data collection in order to optimize the quality of information of models were provided, based on the simulations.

Keywords: geostatistics, plastic debris, spatial mapping, microplastic abundance characterization

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## The OceanWise European project

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Expanded polystyrene (EPS) is a plastic foam usually used globally in packaging and insulation including as food trays, drink/food containers and fish boxes. Different properties of EPS explain its very wide use in the industrial world. EPS weighs very little compared to its volume, which makes it very cost-effective in terms of transport compared to other packaging options. Moreover, it is also deemed safe for use with food. It also has very good insulating and mechanical properties that facilitate the transport of foodstuffs. Nevertheless, this massive use of EPS has consequences for the natural environment. Although EPS is recyclable, it seldom is, because of its contamination by food or because of the low cost-effectiveness of transporting and recycling



expanded material. As a result, it is often used to produce energy or landfilled. Its lightness means EPS is easily blown from garbage bins and landfills. It is not biodegradable and tends to fragment, with small pieces travelling long distances. It can become marine litter and break down into microplastics that persist in the marine environment and contaminate the food chain. Thus, particles are commonly found in the marine environment of the Atlantic coast and ocean. It is of particular concern because of the large quantities found on beaches and the potential hazard it poses to marine environment. OceanWise aims to jointly develop a set of long-term measures to reduce the impact of EPS in the North-East Atlantic Ocean. Tangible solutions will be set by addressing the entire life-cycle of EPS products to achieve transnational sound management of EPS marine litter in the Atlantic. This challenge will be address by developing knowledge to reduce the impacts of EPS items and by raising the ability of competent authorities and key sectors across the Atlantic region to implement more sustainable management options.

Keywords: Expanded polystyrene, Plastic pollution, Atlantic sea, impacts of EPS, sustainable management

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## Plastic fluxes toward the oceans, contribution of the Schelde River (Belgium).

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Plastic waste in coastal areas and the expanding “Plastic Soup” in our oceans are a growing threat for the marine environment. In recent years the role of rivers as a potential main contributor to marine plastic pollution has been suggested. Yet, the scale of such input remains to be systematically quantified. Also the understanding of seasonality remains poorly studied. If high contributions can be determined, considering the vastness of oceans and seas and the great depth of some, the feasibility of projects removing plastic in rivers might be better than those in which plastic is removed from the open oceans. Therefore, the University of Antwerp (ECOBE & SPHERE) will study the potential contribution of the Schelde river to the worldwide “Plastic Soup”. The aim is to quantify the plastic flux for the entire Schelde basin. Are there areas where plastic is retained? What are the biggest sources? What is the retention time? In this study we will only focus on macroplastics ( $\geq 2.5$ cm). For this fraction it is still realistic to design removal strategies, which eventually is the ultimate goal of this project. Also, a large fraction of microplastics originate from disintegration of macroplastics. Therefore this project is thought to have a beneficial impact in

microplastic pollution as well. To answer the above mentioned questions, in 2018 a monitoring network is set-up. Fluxes will be determined by point measurements. Different monitoring techniques will be used, for instance: the use of fyke nets, a fishing technique called anchor netting, the use of a specially designed mobile sampler and a number of Trash Interceptors that will be placed in the Schelde and its main tributaries. Additionally a ‘Citizen Science’ project is set-up to analyze plastic waste that ends-up on the riverbank. This might yield vital information on its sink function.

Keywords: Schelde, River, Estuary, Freshwater, Plastic Fluxes, Macroplastic, Catchment

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## The Baltic Sea in the microplastic research map: a model from sources to sinks

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The Baltic Sea is the largest Brackish Sea worldwide, displaying a unique salinity gradient and sensitive ecosystem. It is under severe anthropogenic pressure, including the pollution by plastics. At the Leibniz Institute for Baltic Sea Research Warnemünde (IOW), a number of projects are currently investigating plastic and microplastic (MP) pollution dynamics and increase the prominence of the Baltic Sea in the global MP research map. MP sources are being investigated, such as wastewater treatment plants, including those that use fine-scale filtration as potential trappers of MP loads (Plastrat project). Diffuse MP sources (i.e. agricultural soils) are being explored in a large project (MicroCatch Balt), focusing on the Warnow as an exemplary river system. Multiple efforts are being taken to sample, isolate, and model dynamics of MPs in the riverine/estuarine ecosystem; resulting models potentially being comparable to other systems globally. When MPs reach the sea, the BONUS MICROPOLL project is then assessing a multitude of research questions towards MP and associated pollutants. What plays an important role here is MP-biofilm dynamics, a research area that initially established the IOW in the MP research map. All projects are facing the associated methodological challenges of extracting MPs from notoriously

difficult sample mediums such as sludge or river mud, and methodological improvement is a significant part of our research. Progress has been made to improve the reliability of both sampling and sample processing, with strides being made to examine microplastics down to low sizes ( $\sim 10 \mu\text{m}$ ; in association with the Leibniz Institute for Polymer Research, Dresden) while examining considerable sample volumes (200-1000 L). Thus, the IOW is working to improve the methods of MP science as well as both deepening the understanding of the Baltic Sea's place in the global microplastic research map and increasing the understanding of MP dynamics from source to sea.

Keywords: establishing microplastic sources, sediment samples, water samples, biota samples, marine litter

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## PAEs level in European sea bass *Dicentrarchus labrax* experimentally exposed to PVC microplastics

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Release of hazardous chemicals and plastic additives from ingested plastic debris constitute a potential threat to marine organisms, which needs to be investigated in particular fish species of high value for human consumption. Among plastic additives that recently attracted the attention of the scientific community there are phthalate esters (PAEs), which can have various noxious toxic effects on organisms, in particular, they are able to act as endocrine disruptors (EDs) even at very low concentrations. This study investigates, for the first time, the presence of eight different phthalates (MBZP, MBP, MEPH, DNHP, BBzP, DEPH, DIOIP and DNDP) in muscle of European sea bass *Dicentrarchus labrax* chronically exposed to microplastics. The fish were exposed for 90 days to three different treatment diets: food pellets as control (CTRL), food pellets supplemented with native  $300 \mu\text{M}$  PVC microplastics (0.1% w/w 0.3 mm PVC; MPV), food pellets supplemented with polluted microplastics incubated for three months in the waters of Milazzo harbor (0.1% w/w 0.3 mm PVC; MPI). After 30, 60 and 90 days, fish were sacrificed, and muscle stored for subsequent analysis at  $-80^\circ\text{C}$ . Taking into account the ubiquitous nature of plasticizers stringent laboratory and sampling procedures were taken to ensuring the integrity of results. The DIOIP concentrations were below the detection limit in all sample, with regard to the detected PAEs, results evidenced a

different fingerprint among the different treatments. Muscles of fish treated with MPI show higher PAEs concentration than MPV and control and seem to decrease with increasing time of exposure. DEHP was the PAEs with highest frequency of detection and its concentrations ranged from 1.10 to 3.56 µg/g d.w. This compound is used as a plasticizer in polymer products, and it represents more than 95% of the total use of PAEs in Europe, mainly in flexible PVC.

Keywords: Phthalates esters, Plastic tracers, Endocrine disruptors, Sea bass

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## Review on microplastic studies in Brazilian aquatic ecosystems

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The microplastic studies in the world is increasing, reflecting concern about its effects on the ecosystem. The same happened in Brazil, especially in the last six years, but few places were monitored. Thus, a literature review was conducted to collect data on microplastic pollution in Brazilian aquatic ecosystems, analyzing this pollutant in samples of sandy and muddy sediments, plankton and other invertebrates and vertebrates. As results, we highlight the following points: 56% of the studies were published in the Journal Marine Pollution Bulletin; collaborative and independent studies presented the same number; the Brazilian Northeast and Southeast were the most studied areas; the investigation of microplastics associated with biota was highlighted (43% of studies) and only one study was conducted in a freshwater environment. While recognizing the relevance of the problems caused by pollution, it is still difficult to trace the distribution, monitoring and consequences of microplastics in Brazil, because studies are relatively recent and still scarce when compared to other regions of the planet, demonstrating the need of new investigations. More extensive investigations have been carried out in areas of the Northeast and Southeast coast, mainly in the states of Pernambuco and São Paulo, there is a need to encourage the formation of new groups outside this regions. In addition to the coastal area, monitoring in freshwater ecosystems deserves attention, since only one of the studies was carried out in this environment. The present study suggests the proposal of new studies that deepen the subject, since many gaps need to be fulfilled. In the face of the harmful landscape resulting from misuse and urbanization, research is important to assess risks and impacts, and to improve the monitoring of the sources of these wastes

in order to contribute to public awareness, environmental planning and coastal zone management processes.

Keywords: Brazil, literature review, microplastic

\*Speaker

## Hangin by a fibre: the risk of sample contamination by airborne plastic fibres

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Fibres originating from clothing and other fabrics are ubiquitous in the environment. Studies focusing on microplastic ingestion by fish and invertebrates have sometimes reported 100% of the ingested plastics as fibres. However, recent studies show that in common lab facilities it is impossible to exclude contamination of samples through airborne fibres. Scientists must be aware of this so as to not overestimate the extent of plastic ingestion by (marine) organisms. So far scientists developed different methods to cope with potential contamination with varying results.

Keywords: microfibrs, standardizing protocol, secondary pollution

\*Speaker

## Microplastic particles in atmospheric deposition of the metropolitan area of Hamburg, Germany

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The input of microplastic particles via the atmosphere has been rarely investigated, so far. Here, we present results on microplastic concentrations in the atmospheric deposition in the metropolitan region of Hamburg. In total, six investigation sites were equipped with three bulk precipitation samplers each and sampled over 12 weeks biweekly (12/17-03/18). Three sites were located in the rural area south of Hamburg comprising one open field site and two throughfall sites under beech/oak and douglas fir forest canopy, respectively. Three further sites were selected within the city following a transect from North to South representing urban sites of varying degrees

concerning population, traffic and industrial pressures. The resulting 108 samples plus 6 procedural blanks were pretreated with sodium hypochlorite (7- 14 %) in order to destroy biological organic material. The samples were transferred to cellulose filters (VWR 616-0812) and underwent staining with Nile red solution subsequently. Particles and fibers were counted under UV light within a photo box and under a fluorescence microscope (AxioLab A.1, Zeiss). Results show that microplastic particles are ubiquitous at all sites. In contrast to other studies (Dris et al. 2015, 2016; Cai et al. 2017), fragments were significantly dominating compared to fibers. The spatial distribution comparing the urban sites concentrations followed in the order from high to low "center" (University; large population density) – "north" (Henstedt-Ulzburg, low population density, suburb) – "south" (Wilhelmsburg, middle population density, port and industrial facilities) with highly varying concentrations within the time series. Surprisingly, the rural sites in the southern part of Hamburg showed highest concentrations (Douglas fir > open field > beech/oak). This result is most likely a result of factors such as the comb out capacity of the different forest types and/or direct input pathways from the agricultural areas and the nearby highway.

Keywords: microplastics, atmospheric deposition, open field vs. throughfall precipitation

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## Marine Microplastic: Realistic test materials for laboratory studies of ecosystem impacts

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Studies investigating the possible effects of plastic litter on marine biota have almost exclusively utilised pristine plastic materials that are homogeneous in polymer type, size, shape and chemical composition. This is particularly the case for microplastics as collecting samples of such material from the marine environment in quantities sufficient for use in laboratory impacts studies is simply not feasible. Crucially, weathered plastics collected from the marine environment show considerable physical and chemical differences to industrial plastics. In the current study, we describe the preparation and characterisation of a more environmentally realistic marine litter-derived microplastic reference material ( $\leq 3$  mm) for use in fate and effects studies. Weathered marine plastic litter (351 items) was collected from the coast of Texel (Netherlands) identified and categorised (fibre-based, packaging, foam, plastic boxes and jerry cans, bottles, gloves and miscellaneous plastic materials). Ropes, nets and sheeting comprised ~ 70% of the collected material,

which contained 9 different polymer types. The macroplastic material was sub-sampled and subjected to a cryo-milling and sieving process to create the microplastic reference material. To confirm that the original macroplastic polymer distribution was mirrored in the generated microplastic sample, it was subjected to ATR-FTIR and DSC analysis. Particle size distribution (PSD) of the microplastic, showed that 68% (by mass) of the particles were in the range between 0.5 and 2.0 mm. Particle number increased with decreasing particle size fraction. Scanning electron microscopy revealed a wide range of particle sizes and shapes reflecting the properties of the different polymers. ICP-MS and ICP-OES analyses revealed the presence of a broad range of elements (e.g. Al, Cr, Fe, Mg, Pb, S and Zn) associated with the final sample. The additive organic chemical profile of the microplastic mixture was also determined by GC-MS analysis. A broad range of plasticisers, stabilisers, antioxidants and flame retardants were identified.

Keywords: Microplastics/Realistic test materials/Laboratory studies/Additives

\*Speaker

## Thermoanalytical methods for the optimisation of microplastic detection in freshwater sediment samples

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During the last decades, global awareness of the ubiquitous abundance of microplastic (MP) in the environment increased along with publications on MP in water and sediments. However, the presented results are hard to compare due to different sample pre-treatment techniques and detection methods. Furthermore, most of the analyses are very time consuming. Therefore, the approach of our group is to develop systematic and fast methods for MP identification and quantification in water and freshwater sediment samples using thermoanalytical methods - here exemplified by investigations of limnic sediments of different depth zones of the urban Lake Tegel in Berlin, Germany. In the present work the samples were collected by a transect method using a sediment hammer corer. The following two-step method has proven to be effective: First, a sample pre-treatment by density separation was performed using sodium polytungstate solution (1.7 g mL<sup>-1</sup>) combined with additional processing (wet sieving, ultrasonic bath, shaking, centrifugation, freezing and filtration) to concentrate the organic matter, including potentially contained MP. For the validation of the density separation, thermogravimetric analyses (TGA) of the samples (without density separation, light fraction, residue) were carried out. Secondly, the light fraction was

analysed using thermal extraction desorption gas chromatography mass spectrometry (TED-GC-MS). This was performed to subsequently analyse specific degradation products of respective polymers for MP identification and quantification, as well as for the characterisation of the environmental matrices. The TGA-results of the developed routine showed a successful concentration of light fraction and separation of inorganic matter. Therefore, TGA can function as an effective method control for sample pre-treatment. Additionally, the TED-GC-MS results detected the abundance of MP in different concentrations throughout all depth zones of the studied lake basin. Furthermore, the sampling points of the profundal zone were identified as hotspots for MP accumulation.

Keywords: freshwater, lake, sediment, thermoanalytic, TGA, TED, GC, MS, density separation, SPT

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## Sublethal effect of cadmium in *Donax trunculus* (mollusca, bivalvia): Variation in vitellogenin rates during the exposure and depuration period

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In recent decades, anthropogenic activities (pollution of the marine environment, irresponsible fishing, anarchic urbanization of the coast, etc.) have made Mediterranean ecosystems dangerously vulnerable. Contamination of aquatic ecosystems with foreign substances remains a serious environmental problem of increasing concern. Used in most industrial processes, these toxic substances are then released via the effluents into the receiving medium often without prior treatment. Indeed, previous work has shown that the Gulf of Annaba (Algeria) is affected by several pollutants, various heavy metals were particularly detected in the sediments and tissues of an abundant edible species of the bay *Donax trunculus*. Our present study aims to evaluate the impact of metallic pollution by using a heavy metal frequently encountered in the Gulf of Annaba (Algeria): cadmium, on the levels of vitellogenin (precursor of vitelline) in *Donax trunculus* during the period exposure and depuration. All the results show a highly significant increase in vitellogenin levels ( $p \leq 0.001$ ) for the four exposure times. During the restoration period there is generally a gradual recovery of these rates. These results suggest that this xenobiotic disrupts the endocrine system of *Donax trunculus* by stimulating certain endogenous factors (estrogen hormones) in the



induction of vitellogenin.

Keywords: Donax trunculus, Vitellogenin levels, Cadmium, Pollution.

\*Speaker

## Introducing quality criteria for the analysis of microplastic in biota samples

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Data on ingestion of microplastics by marine biota are quintessential for monitoring and risk assessment of microplastics in the environment. Current studies, however, portray a wide spread in results on the occurrence of microplastic ingestion, highlighting a lack of comparability of results which might be attributed to a lack of standardisation of methods. We critically review and evaluate recent microplastic ingestion studies in aquatic biota, propose a quality assessment method for such studies, and apply the assessment method to the reviewed studies. The quality assessment method uses ten criteria: Sampling method and strategy, Sample size, Sample processing and storage, Laboratory preparation, Clean air conditions, Negative controls, Positive controls, Target component, Sample (pre-)treatment, and Polymer identification. The results of this quality assessment show a dire need for stricter quality assurance in microplastic ingestion studies. On average studies score 7.8 out of 20 points, indicating that information is missing which reduces reproducibility and reliability of the individual studies.

Keywords: microplastic, quality assessment, review

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## Study of Microplastic formation: Accelerated degradation of polyamide 6 in thermal and hygrothermal environment

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Pollution of marine environment by microplastics, which are particles with size inferior to 5 mm [1], has become a global major concern [2, 3]. The aim of the present study was to understand the formation of microplastics in accordance with a fragmentation process. Several thermoplastic polymers were used, in particular polyamide 6 was investigated. Aging was accelerated by raising temperature. Aging was carried out on 200 µm thick film under different conditions: water deoxygenated environment in a stainless steel vessel, thermo-oxidative environment in an oven and coupling of both to simulate real conditions. Physico-chemical and mechanical characterizations were performed in order to investigate modifications at different scales. Results revealed a decrease of molecular mass and an increase of crystallinity. Spectroscopy showed the formation of carbonyl groups. Tensile tests revealed an embrittlement, characterized by a lower stress at break corresponding to a ductile-brittle transition. The ability to form microplastics was tested on aged samples introduced in a beaker on a rotary shaker to correlate the embrittlement with the fragmentation of the material.

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Keywords: degradation, fragmentation, microplastic, embrittlement

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## Macro-plastic occurrence and flows in large European rivers draining the Mediterranean Sea: the Rhône (France)

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The Mediterranean Sea has been documented to be largely impacted by plastic, being nowadays one of the major hot spots in the world. Rivers are considered important input pathways of plastics to coastal areas. However, the occurrence of plastic fragments in rivers and derived fluxes are poorly understood. In order to shed light on this key issue, the Joint Research Centre (European

Commission) launched in 2016 a riverine litter observation network within the RIMMEL project. The main objective of this project was to quantify the floating macro litter loads through rivers to marine waters, combining the experimental observation of macro litter and the modeling of their loading. Floating macro litter (including macro-plastics) was monitored in more than 50 European rivers during 2016-2017. We present here results from the Rhône river (Switzerland / France), one of the major European rivers. Monthly observations were performed in Arles (Southern France), around 40 km NW from the river mouth in the Gulf of Lion (NW Mediterranean Sea). Results showed a mean flux of ~50 litter items/hour (belonging to 22 macro-litter categories) floating down the Rhône to the Mediterranean coast, with plastic items representing the 77 % of macro-litter, supporting the hypothesis of rivers as key pathways for plastic litter to the marine environment. The most abundant plastics were: pieces, bags, sheets and bottles (Fig. 1). Considering the median value as a constant flux rate, a rough estimation of the annual input of floating macro-plastics from the Rhône to the NW Mediterranean would be about 220 000 items (with an important uncertainty associated due to the high temporal variability). This is a lower-end estimation since only floating items were counted. These macro-plastics are expected to eventually become micro-plastics due to environmental aging, with yet unknown implications for the aquatic ecosystem.

Keywords: marine litter, riverine litter, plastics, chemical pollution, Mediterranean Sea

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## Microplastic pollution in aquatic environments of Estonia.

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Our study presents the abundance, distribution and characteristics (type, shapes, colour and size) of microplastic (MP) in Estonian waters of the Baltic Sea and freshwater environments. The selection of sampling areas in the coastal sea was based on the locations of the potential MP pollution sources – rivers, wastewater treatment plant (WWTP) outflows. Besides this open sea areas were sampled to assess the broader distribution of MP in sub-basins around Estonia. Water samples from the surface layer were collected at 8 regions in 2016–2017 using a 333 µm mesh manta trawl. Microplastic pollution levels in sediments were investigated in 2017 in the Gulf of Finland. Samples (upper 5 cm) from 6 locations were collected using Van Veen grab and Gemax corer. The results showed the presence of MP in each water sample collected, MP made up approximately 30% of the total microlitter particles in both sea surface layer and sediment samples.

MP pollution in sea sediments and surface waters was highest in the central part of the Gulf of Finland. The presence of fibres (both plastic and non-plastic) prevailed over particles; the prevalent colour of fibres was blue and black. Our study also includes characterization of MP in urban and rural rivers, identifying key sources of MP to the investigated freshwater systems. Samples were also collected at the biggest WWTP of Estonia from treated sewage water before outfall tunnel using Manta trawl cod end (mesh size 333 µm), the same method was used for river studies. The results indicate that the WWTPs can be considered an important source of the MP in the Estonian waters. Our future research will focus on terrestrial sources of the MP (including industrial hot spots and stormwater systems), and on the amounts of MP on marine biota – benthic animals, and pelagic and benthic fish.

Keywords: microplastic, Baltic Sea, sediments, sea surface, WWTP, rivers

\*Speaker

## Litter rim of the Baltic Sea coast: monitoring, impact, and remediation

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Application of the unified methodology to monitoring of micro-, meso-, and macro-litter along the Russian, Estonian, and German marine beaches of the Baltic Sea is the main goal of a new project co-funded by the Russian Foundation for Basic Research, the Estonian Research Council, and the German Federal Ministry of Education and Research in frames of the ERA.Net RUS Plus Call 2017 - Science & Technology (project number 429). These unified methods will also be tested on the coasts of lagoons, estuaries, and river mouths of the partner countries. Migrations of marine litter between the beach and underwater slope will be investigated, with emphasis on observations of massive litter beaching after stormy events. Link between marine litter and human pathogens will be examined. An impact of presence and migrations of litter in sea coastal zone on benthic ecosystems in coastal marine environment will be investigated in microcosm experiments. Numerical modeling will be applied to relate the amount and distribution of the beached litter to hydrophysical and meteorological conditions, allowing for the development of a new monitoring methods and a strategy for more effective cleaning of the sea coastal zone. The expected results include (i) implementation of the unified methods of the beach litter monitoring on the marine coasts of the

three partner countries; (ii) results of testing of this methodology for coasts of lagoons and river mouths/estuaries, with development of specific recommendations; (iii) development of physically-based strategy for cleaning of the coasts after stormy events; (iv) results of investigation of functioning of microbial biofilms on different sorts of plastic, disclosing the relationship between litter and human-pathogens; (v) results of mesocosms experiments on effects of marine meso and macrolitter on benthic ecosystem.

Keywords: beach litter, microplastics, monitoring, storm, national park beaches, human pathogens, mesocosm experiments, the Baltic Sea

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## MICROPLASTICS IN THE MARINE ENVIRONMENT: IMPLICATIONS ON COMMERCIALY IMPORTANT FISH AND INVERTEBRATE SPECIES OF THE EASTERN AEGEAN SEA.

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The occurrence of microplastics in the marine environment has increased over the decades, raising concerns about the effects of this contaminant on ecosystem functions and human health as current knowledge is limited. The purpose of this study is to offer an assessment into microplastics and their distribution throughout the marine trophic chain. The gastrointestinal tracts of 66 marine specimens were collected and analysed between the months of February-April 2018. All species analysed inhabited different water columns across the eastern coast of Samos Island, in the Eastern Aegean Sea. These included eight commercially important fish species (*Sparus aurata*, *Sarda sarda*, *Sphyraena viridensis*, *Boops boops*, *Diplodus annularis*, *Serranus cabrilla*, *Trachurus mediterraneus*, *Mullus barbatus*) as well as four invertebrate species (*Paracentrotus lividus*, *Todarodes sagittatus*, *Parapenaeus longirostris*, *Ostrea edulis*). All individuals exhibited microplastic contamination, with a total of 2,725 microplastic items identified among the 66 examined specimens. The abundance and prevalence of different types of microplastics were compared throughout all specimens collected. Plastic fibres were ubiquitous throughout all samples analysed and accounted for 76% of all microplastics detected in this study. A significant variation in microplastic abundance across four main water column levels was recorded (Figure 1), with pelagic, omnivorous fish species reporting a consistently higher number of microplastic items than both demersal fish and benthic

invertebrate species (one-way ANOVA:  $F(3, 62) = 7.896, p = 0.000153$ ). The results of this study prove the persistence of microplastics throughout the marine trophic web. Since three billion people rely on the ocean as their primary source of protein, it is crucial to spur further research efforts to investigate the unknown consequences of microplastic contaminants. Such pollutants can serve as vectors and aid in the transference of harmful chemicals, emphasising the need of more sustainable alternatives in order to safeguard marine life as well as the health of seafood consumers.

Keywords: Microplastics, Pollution, Water column, Marine trophic chain, Aegean Sea, Human health, Gastrointestinal analysis

## Variations in phthalates concentration present in fin whales (*Balaenoptera physalus*), gray whales (*Eschrichtius robustus*) and humpback whales (*Megaptera novaeangliae*) in Mexican Pacific

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Over last decades, plastics debris have become a major problem in the marine environment due to its persistence and chemical properties. The fragments derived from the larger plastic can be easily incorporated in food web of marine organism and its added toxic chemicals are incorporated in their tissues may cause long-term adverse effects. As an example the phthalates considered endocrine disrupters, can interact with hormone synthesis and alter reproduction or other physiological and metabolic functions. The purpose of this research is to determine concentration of phthalates present in three large whale species (*Balaenoptera physalus*, *Eschrichtius robustus* y *Megaptera novaeangliae*) from its economic, social and environmental importance to Mexican community. In this way we will use this chemical such as plastic tracer in these different species. The sampling shall be carried out according its principal areas of reproduction, breeding or feeding; San Ignacio Lagoon (gray whales), Los Cabos (humpback whales) and La Paz Bay (fin whales). Biopsies of 30 individuals for each species will be taken (90n). Therefore, extraction and analysis of phthalates will be carried out (DEHP and MEHP). The generated information will allow to establish a platform for a research field that is in development, as well to know the grade of impact caused by microplastics to which these marine organisms are exposed in the different regions.

Keywords: Microplastics, plastic additives, baleen whales, Mexican Pacific

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## Modeling approach of micro- and nanoplastics in aqueous and cell environments

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*Micro- and nanoplastics are tiny plastic particles with size less than 5 mm and 0,1 µm, respectively, and they come from a variety of sources, including degradation of larger plastic fragments and direct release of micro- and nanoparticles from household and customer care products. Microplastics is a global pollutant that may be affecting the behavior of marine and freshwater ecosystems. From aquatic ecosystems microplastic can end up to food webs, foodstuffs and tap water which could potentially increase exposure of chemicals to humans and thus can be risk to human health. However, the human health effects are still unknown. Based on chemical analysis (NMR, MS, FTIR) of microplastic samples, molecular modeling tools can be used to study whether micro- and nanoplastics could pass through cell membrane or bind to transporters that can be used for estimating their effects on human. Molecular modeling allows also to explore the role of plastic additives and organic pollutants in micro- and nanoplastics. The experimental assays will be applied for monitoring cell viability via metabolic activity after micro- and nanoplastic treatment. By combining modeling together with toxicity studies, we can build predictive models to estimate toxicity of micro- and nanoplastics. Our strength in UEF Kuopio Campus is to combine efficient analytical tools with molecular modeling, medicinal chemistry and toxicology. This will offer a novel insight on the toxicity of micro- and nanoplastics and their impact on human health.*

Keywords: Microplastic, Nanoplastic, lake, marine, cell, molecular modeling, simulation

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## Ingestion of plastic debris (Macro and micro) by longnose lancetfish (*Alepisaurus ferox*) in the North Atlantic Ocean

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Pollution by plastic marine debris constitutes a major threat to marine life. Ingestion of plastic marine debris by seabirds, turtles, and marine mammals is well acknowledged and has been recognized as a serious hazard to marine biota. The detrimental effects of plastic debris on marine biota include physical entanglement, decreased nutrition from intestinal blockage, and suffocation or decreased mobility. Despite the prevalence of studies documenting the environmental implications of plastic debris in the world's oceans there have been few reports of plastic ingestion by large marine fishes. Reports of plastic ingestion in larger, higher trophic level pelagic fishes are sparse in

the literature but include reports from species such as dolphinfish, tunas, and moonfish.

Longnose lancetfish, *Alepisaurus ferox* Lowe, 1833, a piscivorous ambush predator, is one of the most common bycatch species of the tuna longline fishery. It has a worldwide distribution extending from 45°N to 45°S in the open ocean pelagic ecosystems of the world oceans.

This study evaluates the ingestion of plastic marine debris by *A. ferox*, with the goal of quantifying the amount and type of plastic marine debris ingested. As far as we are aware, there has been no detailed report on plastic ingestion of this fish in the North Atlantic Ocean and it's the first time that microplastics are studied in the stomachs. The stomachs of the fish examined contained many pieces of synthetic materials such as polyethylene and vinyl in addition to ordinary food items (e.g., fishes, cephalopods, shrimps, salps, Pyrosoma). Our study shows that plastic ingestion in *A. ferox* is more prevalent than previously suggested. This study adds to the body of knowledge on piscivorous fish ingestion of marine debris.

Keywords: macroplastics, microplastics, pollution, *Alepisaurus ferox*, North Atlantic Ocean

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## Influence of solar radiation and plastic type on the bacterial community composition of plastic's biofilm in the North Adriatic Sea

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Once plastic enters the ocean it rapidly becomes colonized by a biofilm. Factors shaping the biofilm's community composition and development are still unclear. In this study we aimed at understanding how solar radiation and plastic type might influence the biofilm's bacterial community composition. We incubated high and low density polyethylene (HDPE and LDPE), polypropylene (PP), polyvinyl chloride (PVC) with two different additives (DINP and DEHP), and glass serving as an inert control over a two-month period in the North Adriatic Sea under minimal light and ambient solar radiation. Samples were taken after one week, one month and two months and the bacterial 16S rRNA gene was used to determine the biofilm community composition. Significant differences were found between the community composition of the biofilm on samples kept under minimal light conditions and ambient solar radiation, with high abundance of cyanobacteria in the ambient solar radiation treatment contributing largely to the variability in bacterial community composition in LDPE, HDPE, PP and glass, especially after one week and two



months of incubation. On both PVCs, however, cyanobacteria were much less abundant, and differences between minimal light and ambient solar radiation conditions were largely due to other taxa, such as the highly abundant *Alteromonadaceae* in the ambient solar radiation treatment after one week incubation, and relatively higher abundance of *Cellvibrionaceae* in the minimal light treatments after two months of incubation. Within treatments, differences between the PVCs and the other surfaces were highly significant over the whole sampling period. However, bacterial families commonly forming biofilms, such as *Rhodobacter*, *Hyphomonadaceae* and *Flavobacteriaceae*, were relatively abundant in all plastics. We therefore suggest that the community composition of plastic's biofilm is shaped by the interaction between normal biofouling processes, environmental factors, such as solar radiation, and plastic type related properties.

Keywords: biofilm, plastisphere, bacterial community, bacteria

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## Characterisation of microplastics (floating and ingested by mussels) in the harbor of Marseille (French Western Mediterranean)

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The FUI Microplastic2 project aims to assess the contamination of the environment by microplastics (MP), as well as micro-pollutants and micro-organisms associated with them, on river systems, estuaries and harbour. Within the framework of this project, several goals were addressed, including the efficiency of sewerage networks and treatment plants, the origin of MPs, the modelling of their dispersion and a better understanding of harm to biota. In addition, one of the challenges was also to develop techniques and methods for the chemical and isotopic characterisation of MP, in order to perform a more complete diagnosis of MPs. First, the goal was to characterise MPs by number, weight, nature (fragments, fibers, pellets, films, and polystyrene), polymer types (PE, PP, PA, PS, etc.) and to characterize the associated zooplankton. The city of Marseille located in the North West Mediterranean and south of France was selected as a study site because of its size

(second largest city in France in terms of population), its urbanization (French third urban area) and its harbour (main harbour in France). The preliminary results on midterm assessment (2018) for this 3-year-project (end of 2019) are presented in this poster. The average microplastic concentration was ranging from 39 199.57 items/km<sup>2</sup> in 2016 to 191 944.44 items/km<sup>2</sup> in 2017, with a maximum of 768 800.00 items/km<sup>2</sup> (spring 2017). A difference in concentration was observed between winter and spring. The MP concentrations were found to vary along the bed of the sewage outfall, showing its influence. The associated fauna was mainly composed by small copepods *calanoida* with the same size pattern as microplastic but also *cladocera* and *appendicularia* groups. The analyzed microplastics were mainly composed of PELD (22%), HDPE (16%), PP (8%) polymers. MP ingested by mussels were composed by fragments (56%), filaments (28%) and fibers (16%).

Keywords: microplastics, identification, micro, pollutants, micro, organisms

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## Actions of a marine natural Park in France to reduce impact of marine debris and microplastics in Iroise sea.

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The Iroise marine natural park was created in 2017 and the management plan of the Park, adopted in 2010, recommends a reduction of the maritime and ground pollutions. For marine debris, the marine Park realizes a follow-up since 2010. From the protocol OSPAR, 4 sites allow to estimate the impact of marine debris in Iroise and since 2017, this marine area protected surveys microplastics. In 2018, we realized a first review of these surveys. Contrary to the national data, 70 % of marine debris are a marine origin in Iroise. A lot of types of marine debris were identified, in particular the professional fishing. It is the activity which contributes most to this pollution (13 %) with the rope, the nets of various diameters, fishing lines (angling) and pieces of crab/lobster pots. So, it represents an annual volume 148000L on the coast of Finistère (Britannia). The first results on microplastics show that the impact in Iroise is also important and that the ground contributions are important.

To reduce these ground and maritime contributions and to avoid that this marine debris becomes microplastics, the marine Park established several actions in partnership with the municipalities of

the coast and the fishermen. So, interventions in the maritime high schools for the futures fishermen are led to make sensitive them in these pollutions and in the consequences. Actions in ports are proposed to get back marine debris coming from boats and by valuing fishing nets. Finally, for the ground contributions, the marine Park suggested buying tubs on beaches to get back marine debris and to avoid that they return to the sea. The marine Park launched an awareness campaign for the inhabitants of the littoral municipalities. This campaign is realized on the evacuation of rainwater to avoid that people throw waste towards the sea.

Keywords: marine debris, marine park, rope, net fishermen

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## The microplastic abundance in surface water increases and the size of plastic particles decreases near urban wastewater and stormwater sources in Lake Kallavesi, Finland

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The effects of microplastic pollution in freshwater basins remain generally understudied. The main aim of this research is to focus on the size and distribution of microplastics released from urban stormwater and wastewater into Nordic freshwater ecosystems such as Lake Kallavesi, Finland. Two sampling methods, a pump collecting different size fractions of 20, 100 and 300  $\mu\text{m}$  and a common manta trawl (333  $\mu\text{m}$ ), were used to collect surface water microparticles from 12 sampling sites at Lake Kallavesi, Finland. The sampling sites represented various potential sources of microplastic pollution, such as the city harbour, a highway bridge, a discharge pipe of a wastewater treatment plant (WWTP) and a snow dumping site. FTIR microscopy was used to determine the polymer type of plastic particles. The total average concentration of microplastic particles in manta samples was 0.27 particles  $\text{m}^{-3}$ . The number of microplastics was significantly higher in smaller size fractions obtained with pump collection (averages 155, 12 and 1.8 particles  $\text{m}^{-3}$  for sizes 20, 100 and 300  $\mu\text{m}$ , respectively). The results indicated that larger ( $> 300 \mu\text{m}$ ) microplastics were abundant in the city harbour, whereas smaller microplastics had several sources, such as the snow dumping site and the WWTP. The majority (64 %) of the identified microplastics

were fibres. All identified microplastics were common polymers, such as PP, PE and PET, but there was no clear correlation between the material type and the sampling site.

Keywords: freshwater, lake, stormwater, wastewater, microplastic

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## Perception and consumption of plastic: A case study to provide research priority.

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The global world production of synthetic polymer keeps increasing every year and should reach over 335 Million tons in 2018. This massive use of plastic reflects the functioning of our consumer societies which generate need of plastics without considering the ecological impact of their disposal in the environment. Beyond the lack of means and solutions dedicated to plastic waste management, many studies present human activities and in particular household consumption, as a major source of plastic pollution. However, the identification of pollution sources does not provide any information on consumer habits, uses and knowledge to understand the waste stream generated. Yet, such data are required to identify the limits and drivers for a change in behavior regarding the use of plastics. The lack of data on the plastic consumption has led us to carry out a sociological study. We gathered qualitative and quantitative data on the consumption and perception of plastic by individuals. The objective was to evaluate trends in practices and knowledge around plastic and determine the uses and behaviors of individuals according to their socio-demographic and cultural characteristics. Our results reveal different types of behaviors with various level of awareness about the consequences of plastic use and their plastic consumption habits, from Initiated to Saver, Pragmatic and Indifferent. The practices of these different consumers does not seem related to their knowledge of plastics as a material but more driven by their knowledge of the environmental

impacts. Knowledge on plastic is generally poor with confusing notions about the recycling, the biodegradable polymers reflecting a lack of sensitization and education. This study highlights the role of researchers in raising awareness and contributing to a transition towards a plastic-free society. Incorporating Social Sciences in plastic research is, then, of key importance, in order to better understand plastic consumption habits and social behavior.

\*Speaker

Keywords: plastic consumption, social behavior

## Impacts of microplastics on the duckweed *Lemna minor* and potential trophic transfer to the amphipod *Gammarus pulex*

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Microplastic pollution has been widely reported in marine ecosystems all around the world. The intake of marine microplastics has been reported in both wild caught and laboratory exposed species. However, data on the presence of microplastics in the freshwater environment are scarce and the effects on aquatic species are largely unknown. Here the ecotoxicological impacts of plastic microbeads on the freshwater environment were studied. For this, we analysed the potential impacts on two freshwater model species, the duckweed *Lemna minor* and the amphipod *Gammarus pulex*. We also studied the trophic transfer of microbeads from *L. minor* to *G. pulex*. The potential effects of 1 and 45 µm polystyrene (PS) and polyethylene (PE) microbeads on the physiology of the widely distributed *Lemna minor* were firstly investigated. The adsorption of beads per surface area was quantified at four time points by using light and fluorescence microscopy. The fate of 1 µm microbeads within the plant tissue was further examined using confocal laser scanning microscopy. In addition, the growth of *L. minor* following seven-day exposure to PS or PE microbeads was quantified following OECD parameters frond and colony number, root length chlorophyll a fluorescence and plant biomass. Our results showed a strong binding of microbeads to the duckweed surface over time. However, our data indicated no negative effects on the physiology of *Lemna minor*. The strong microbead binding to duckweed tissue suggested *L. minor* as a potential vector

for the transfer of microplastics. Therefore, acute feeding trials under controlled conditions were designed to investigate the microplastic transfer to *G. pulex* as well as potential effects on the amphipods. The parameters measured were mortality, mobility, moulting, % plant biomass consumed, number of beads ingested and excreted and food preference. We hope that our results will help understand the complexity of freshwater microplastics ecotoxicity.

Keywords: Microbeads, Polyethylene, Polystyrene, Freshwater, Aquatic Plants, Amphipods, Eco-toxicology, No Effects  
\*Speaker

## Microplastics in wild mussels (*Mytilus sp.*) from the north coast of Spain: Comparison between digestive treatments

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Microplastic content in mussels (*Mytilus sp.*) from the Ria of Vigo and the coast of the Cantabrian Sea was measured. Additionally, with the aim of comparing the use of acid and alkaline compounds as digestive agents, a comparison of microplastic levels observed in mussels digested with nitric acid and with potassium hydroxide was carried out. The average concentration of microplastics in mussels digested with nitric acid was  $1.90 \pm 2.14$  microplastics/g w.w., being significantly lower than that observed in mussels digested with potassium hydroxide, with a value of  $2.87 \pm 2.81$  microplastics/g w.w. This result confirms that the use of acid compounds like nitric acid as digestive agent induces an underestimation in microplastic levels observed in biological samples; therefore, their use should be avoided in a future standardized extraction protocol. To analyze the spatial pattern of pollution, only the results obtained from the digestive treatment with potassium hydroxide were used. Average concentration of microplastics in mussels from the Ria of Vigo was  $2.30 \pm 1.93$  microplastics/g w.w., while in mussels from the Cantabrian Sea was  $3.44 \pm 3.43$  microplastics/g w.w. Fibers were the most abundant morphotype, followed by fragments. The highest levels of microplastics were not always associated to those areas expected as the most polluted, especially in the Ria of Vigo, where a decreasing trend from the entrance of the ria to the inner zone was observed. Consequently, further research is needed in order to account why the observed pattern of pollution differed from that expected.

Keywords: seafood, biomonitoring, biota samples, bivalve mollusks, rocky shores, digestion method

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# Cross-border assessment of microplastics in seafood and salt

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Presence of microplastics in seafood and salt is already demonstrated within many research projects. Comparing literature data is not always straight forward and regional differences are difficult to assess based on existing data: sample preparation, e.g. gut purification, may be different and analysis methods and quality control measurements are laboratory dependent. It is therefore not clear if consumers of different countries will be exposed to different microplastic concentrations. On demand of the Austrian, Belgian, Danish, Italian and Spanish consumer organization, a comparative study was conducted, analyzing microplastics in different types of mollusks, crustaceans and salts. Microplastics were analyzed by stereomicroscope after KOH- digestion (mollusks/crustaceans), H<sub>2</sub>O<sub>2</sub>-destruction (salt) or sedimentation within polytungstate solutions (clay and silica-containing salts).

For each matrix, difference was made between fibers (Fig., blue), films (Fig., grey) and spheres (Fig., orange). The relative amount of spheres within salt samples was 32%, which was high compared to molluscs and crustaceans, which only contained 4-5% spheres. This difference may be related to the efficient removal of spheres from the digestive tract of the biota.

Effect of different parameters were investigated. Salt samples were grouped depending on the processing method and/or the package material. Highest values, up to  $68.9 \pm 26.0$  microplastics/100g, were found in "fleur de sel" salts, which are harvested at the water surface. Package material did not significantly increased microplastic concentrations. Mollusks were grouped according to species, with maximum concentrations up to  $47.8 \pm 17.0$  microplastics/100g tissue weight. For crustaceans, difference was made between wild and within aquaculture bred crustaceans and between peeled and non-peeled crustaceans.

The omnipresence of microplastics in salt and seafood items studied in this research, again stresses the need of more detailed investigation on human health effects of microplastics.

Keywords: mollusks, salt, crustaceans, cross, border assessment, European consumer organizations

\*Speaker

## Narrative role playing - a science multiplication tool- at the AWI school lab OPENSEA on Helgoland

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Over the last decades there has been an increasing awareness within the scientific community of the importance of communicating science. A growing number of multiple citizen science programs aimed at facilitating the sharing of scientific knowledge with the public. One such approach adopts narrative role plays, involving interactive games which simulate real life situations in a safe environment. During these simulations, participants adopt different roles (scientist, politician) and are posed with a specific research question relevant to e.g. environmental protection. With the AWI school lab OPENSEA we developed a narrative role play on marine litter. In this narrative role playing provides an opportunity at hands-on research and discovery from four different perspectives of plastic pollution. High School students learn about the different perspectives of plastic pollution in the course of different phases through hands-on research and discovery. We apply recent scientific findings and literature as well as, interactive modules designed to take place both on-field and in the lab. The modules are designed to be also applicable for classroom settings which are implemented to the education for sustainable development.

Keywords: Education, science communication, school lab, High School students, plastic pollution

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## A “green” analytical approach for the detection of plastic organic additives and related compounds in marine organisms

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There is no doubt today about the ubiquitous occurrence of plastics in the Mediterranean Sea. Marine organisms can be physically damaged by plastic fragments, but also chemically threaten by their constituents. Organophosphate esters – OPEs and phthalate esters - PAEs are among the most important plastic organic additives. In addition, other compounds of emerging global concern, like



volatile methylsiloxanes – VMSs could be present in plastic materials. In order to investigate their occurrence in marine biota, their food web transfer as well as to enable chemical risk assessment, their determination in marine organisms is needed. We present here a fast and "green" method based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) for the assessment of OPEs, PAE, and VMS in different marine organisms. Our results show that the application of QuEChERS successfully allow the detection and quantification (by gas chromatography coupled with mass spectrometry – GC/MS) of VMSs in *Posidonia oceanica* samples (never reported before) collected in various sites in the NW Mediterranean Sea (Marseille area, France). Interestingly, the highest value (over 400 ng/g d.w. for  $\Sigma 7$ VMSs) was measured in the surroundings of the Frioul Archipelago, a suspected micro-plastic accumulation area. The QuEChERS were adapted and optimized to work with OPEs and PAEs too, resulting in a valid option for these two contaminant classes of emerging concern. Higher levels of phthalates (42 ng/g d.w.,  $\Sigma 7$ PAEs) were found in *Posidonia* compared to organophosphate esters (3 ng/g d.w.,  $\Sigma 9$ OPEs). Our method was also applied for the analysis of fish and mussels from the Mediterranean Sea, confirming the presence of these plastic additives, with slightly higher concentrations in fish for OPEs ( $\Sigma 9$ OPEs=38;  $\Sigma 7$ PAEs=25 ng g<sup>-1</sup> d.w.), but one order of magnitude higher PAE levels in mussels (110 ng/g d.w.) compared to OPEs (11 ng/g d.w.).

Keywords: flame retardants, plasticisers, OPEs, phthalates, siloxanes, QuEChERS, biota

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## Ingestion, Depuration and Translocation Kinetics of Microplastics in Blue Mussels (*Mytilus edulis*)

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We are currently studying the ingestion, depuration and translocation kinetics of microplastics (10 and 90  $\mu\text{m}$ -sized fluorescently labeled polystyrene beads) in blue mussels (*Mytilus edulis*). The purpose of this study was to assess the size-dependent characteristic of ingestion, depuration and translocation kinetics of microplastics in blue mussels. At this time, a preliminary study to determine the optimum tissue digestion method for the tracking of microplastics in the mussels were completed. For this, we artificially injected 10  $\mu\text{m}$ -sized polystyrene beads in the blue mussels (50 particles per animal), digested the tissue of mussels using four digestion methods (i.e., H<sub>2</sub>O<sub>2</sub> digestion, protease digestion, Lipex digestion and KOH digestion), then counted the number of microplastics in digested solution using fluorescent microscope. H<sub>2</sub>O<sub>2</sub> digestion and protease

digestion methods recovered > 97% of injected microplastics while the recovered fraction of injected microplastics by other digestion methods (i.e., Lipex digestion and KOH digestion) were lower than 97%. Because it was reported that H<sub>2</sub>O<sub>2</sub> digestion method has a disadvantage of foam formation during the digestion process that can lower the microplastic recovery, we determined to employ protease digestion method for the detection of the microplastic in mussel tissues. The kinetic study is being conducted by exposing the mussels in artificial seawater containing microplastics (24,000 particles/L) for three days then depurating the microplastics in the mussels for eleven days. We will track the ingestion, depuration and translocation of microplastics in mussels by observing the number of microplastics in gill, digestive gland and non-digestive gland of the mussels. The number of microplastics in hemolymph will be also measured to assess whether the ingested microplastics can be translocated to the circulatory system. We expect that the result of the ingestion, depuration and translocation kinetic study of microplastic in blue mussels can be suggested at the time of the MICRO 2018 International Conference.

Keywords: ingestion, depuration, translocation, kinetics, blue mussels

\*Speaker

## Microplastics as a vector for exposure to hydrophobic organic chemicals in fish; a comparison of two polymers and silica particles, using three different model compounds

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Microplastics (MPs) have the capacity to act as environmental vectors, sorbing and concentrating hydrophobic organic chemicals (HOCs) from the aquatic environment. Current debate as to whether MPs act as vectors for chemical contaminants to aquatic organisms, and whether MPs are a significant pathway for exposure to HOCs compared to naturally occurring particles, requires further experimental evidence. Thus, we conducted an experimental feeding study that sought to assess the relative importance of two synthetic polymeric particles (polyethylene (PE) and polystyrene (PS)) acting as vectors for HOCs in comparison to non-plastic particles (glass particles). By using chemically spiked MPs, we aimed to investigate mechanisms of particle-

mediated chemical transfer and study associated biological effects resulting from particle ingestion in fish. The 3 types of particles (250µm) were loaded with a mixture of model chemicals (benzo(α)pyrene, ethinylestradiol and chlorpyrifos), which differed in hydrophobicity and had 3 distinct toxicological modes of action. Three-spined stickleback (*Gasterosteus aculeatus*) were exposed to 8 different diets: control diets, diets with virgin particles, diets containing particles spiked with a chemicals and, finally, diets loaded with only the chemical mixture but no particles. Fish were fed daily (6 % of body weight and 5 % added particles) for a period of two weeks. Gene expression of well-established biomarkers (CYP1a, ERα, VTG and AChE) was then quantified at mRNA level in the liver and gut. Acetylcholine esterase (AChE) activity was also measured in fish brain. Results showed that all treatments containing chemical mixtures caused changes in gene expression and enzymatic AChE activity. Differences could also be seen between particle types, where PS contaminated particles showed similar pattern with the non-plastic particles, while PE contaminated particles induced lower expression levels, indicating a smaller transfer of chemicals.

Keywords: microplastic, fish, vector effect, chemical contaminants, PE, PS, silica particles

\*Speaker

## Exposure to Polystyrene microbeads induced behavioural changes in *Daphnia magna*

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A growing body of evidence has highlighted that plastic contamination represents a dramatic environmental problem. Plastic wastes contaminate both terrestrial and aquatic ecosystems, threatening all living organisms. Recent studies have focused on the environmental occurrence and concomitant adverse effects of microplastics (MPs). Most of these studies have investigated the ingestion and the toxicity of the main typologies of MPs in marine organisms, but surprisingly data on freshwater species are still scanty. In this work we studied the effects of 1 and 10 µm polystyrene microbeads (PMBs) in the Cladoceran *Daphnia magna*, evaluating the ingestion/egestion capability of daphnids (< 24 h) and adults; assessing changes in individual growth, and investigating PMB-induced behavioural changes, in terms of alteration of swimming activity and reproduction. PMB features were checked and concentration exposures were 0.125, 1.25 and 12.5 µg/L. An initial 24-h uptake/48-h release exposure was performed to assess the ingestion/egestion of PMBs in daphnids and adults and then, a 21-d exposure to evaluate changes in swimming behaviour, in terms of both

horizontal movement and phototactic response. Lastly, we carried out a 21-d reproduction test to assess changes in the reproductive effort. PMBs of both sizes were observed in the digestive tracts of daphnids and adults already after 1 h exposure even at the lowest concentration. Complete egestion did not occur within 48 h, even though the amount of PMBs greatly decreased, mainly in the 0.125 µg/L groups. No changes in body growth were noted, but PMBs ingestion determined an overall significant change in swimming activity and phototactic responses, which compromised reproduction at the highest tested concentrations.

Keywords: polystyrene, ingestion, behavioral effects, *Daphnia magna*

\*Speaker

## Studying (micro)plastics in sediments of Lake Biel, Switzerland

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Since the beginning of the second half of the 20th century, the worldwide plastic production increased from 1.5 Mt/year to more than 320 Mt in 2015 (PlasticsEurope, 2016). (Micro)plastics end up in freshwater bodies and in the marine environment, and are even found in remote places such as the Arctic (Peeken et al., 2018). In undisturbed lake sediments, only selected investigations have been conducted until now.

For this study, a 110 cm long sediment core has been retrieved from Lake Biel in Switzerland. Lake Biel is one of the three large lakes in the Jura region of Switzerland (length of 15 km, width of up to 4.1 km). Its surface area is 39.3 km<sup>2</sup>. The rivers Aare and Zihl/Thielle flowing from Lake Neuchâtel are the main tributaries. The goal of this work is to trace the input of (micro)plastics since the beginning of the plastic production in the 1950s. This should allow an estimation of the continuing input if the production would continue to rise and no measures would take place. Moreover, it contributes to the risk assessment.

For the chronostratigraphy, the sediment core was measured for <sup>137</sup>Cs concentrations revealing peaks of the nuclear weapon tests between 1945 to 1963, the release of the Mühleberg nuclear power station in the middle of the 1970s and the Chernobyl peak of 1986. For (micro)plastic analyses, samples of 2 cm were taken. First, the organic matter was digested then, the (micro)plastic particles were isolated from remaining matrix by density floatation and pressure

filtration. Analysis was done by visual inspection and Fourier-transform infrared spectroscopy. The first results show that only few fibers and small plastic particles are deposited with a decrease in depth. Interestingly, no spheres were found in the layers.

Keywords: Lake, freshwater, microplastics, Switzerland

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## IMPACT OF RECOVERED MARINE DEBRIS: ECOTOXICOLOGICAL ANALYSIS USING MARINE ZOOPLANKTON

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The role of microplastics in the oceans is becoming more relevant day by day. This type of materials, generally sized under 5 mm, belongs mostly to fragmented objects from land-based sources. The aim of this study is to know better the characteristics of the plastics found in the open sea, evaluate their impact and reduce it, principally in the biota. Plastics used were collected in situ by fishermen and brought to port, where the characterization and separation of the items were done. Most abundant items (plastic bags, bottles, nets and industrial packaging) were selected to perform the ecotoxicological bioassays. With that aim, they were micronized to particles below 250 µm and incubated 24 h in a rotatory wheel at a concentration of 100 g L<sup>-1</sup>. Bioassays were carried out with serial dilutions of the leachates obtained. Planktonic species from different trophic levels were chosen: the microalga *Isochrysis galbana*, the copepod *Acartia clausi* and the sea-urchin *Paracentrotus lividus*. For copepods and sea-urchins larval stages were used to increase sensitivity. Population growth rate was recorded for algae, mortality for copepods and larval growth for sea-urchins, and toxicity was quantified using the EC10/LC10 and EC50/LC50 parameters.

Keywords: larval bioassays, leachate, marine debris, microplastics, toxicity, zooplankton

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# Assessment of polystyrene uptake by analytical pyrolysis and relationships with physiological responses in the Mediterranean mussel, *Mytilus galloprovincialis*

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Contamination of marine environments by microplastics (< 5mm diameter; MP) is receiving increasing attention as a main issue of marine litter pollution. MP are ubiquitous, widely interact with living organisms and act as carriers of priority pollutants. Scientific reports agree that MP ingestion may cause gut blockage, altered feeding and energy allocation, with effects on main physiological processes. However, to establish the environmental risk posed by MP and target mitigation measures some issues have to be clarified. Challenges in MP sampling and quantification have not been overcome, and relationships between MP in the environment, their uptake and biological effects need further insights. This study aimed at determining the occurrence of MP of polystyrene (PS) in tissues of adult mussels, along with the evaluation of biological endpoints for mussel physiological status. Mussels were exposed for 4 days to 3- $\mu$ m and 45- $\mu$ m PS-MPs (matching size range of planktonic food sources), both at 1,000 and 10,000 particles/L. Haemocyte lysosomal membrane stability showed the onset of a stress syndrome in mussels exposed to 10,000 particles/L at both MP sizes. A novel method based on analytical pyrolysis was developed. Toluene extracts from alkaline digested samples were pyrolyzed at 500°C; evolved styrene was trapped onto an activated carbon sorbent and determined by GC-MS. Digestive glands showed the highest PS levels at both particle sizes, consistent with MP translocation to different organs through the circulatory system. Along with PS accumulation, gills also showed increased detoxification activity by means of efflux transporters involved in biotransformation pathways. No significant differences in PS tissue concentrations between mussels exposed to 3- $\mu$ m or 45- $\mu$ m MP were observed, suggesting that size influences MP accumulation rate in filter-feeders, i.e. smaller particles may be more effectively translocated to and accumulated in different organs while bigger particles are more effectively eliminated.

Keywords: microplastics, polystyrene, pyrolysis, uptake, lysosome membrane stability, MXR,

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## Microplastic ingestion in fish larvae in the western English Channel

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Microplastics have been documented in marine environments worldwide, where they pose a potential risk to biota. Environmental interactions between microplastics and lower trophic organisms are poorly understood. Coastal shelf seas are rich in productivity but also experience high levels of microplastic pollution. In these habitats, fish have an important ecological and economic role. In their early life stages, planktonic fish larvae are vulnerable to pollution, environmental stress and predation. Here we assess the occurrence of microplastic ingestion in wild fish larvae. Fish larvae and water samples were taken across three sites (10, 19 and 35 km from shore) in the western English Channel from April to June 2016. We identified 2.9% of fish larvae (n = 14 347) had ingested microplastics, of which 66% were blue fibres; ingested microfibers closely resembled those identified within water samples. With distance from the coast, larval fish density increased significantly ( $P < 0.05$ ), while waterborne microplastic concentrations ( $P < 0.01$ ) and incidence of ingestion decreased. This study provides baseline ecological data illustrating the correlation between waterborne microplastics and the incidence of ingestion in fish larvae.

Keywords: Plastic debris Zooplankton FT, IR Coastal Fisheries

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## Reporting on Microplastic in the Marine Environment: Does Coverage in German Print Media meet the Standards of Quality Journalism?

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The contamination of marine environments with microplastics is gaining attention in media. However,

quality of the reporting varies. Among others, speculations about the effects of microplastics may not always be clearly distinguished from verified scientific findings. Additionally, coverage might not refer to its scientific reference nor point out the validity or limitations of its results. In order to evaluate the quality of the reporting we analyzed articles of six German newspapers from 2012 - 2017 including the national "Süddeutsche Zeitung" and "taz" ("Tageszeitung"); two local newspapers ("Hamburger Abendblatt", "Nürnberger Nachrichten"), one tabloid ("Express") and one professional journal ("VDI-Nachrichten of the Organization of German Engineers").

Eleven criteria were evaluated based on the "Media Doctor"-Project of the Technical University of Dortmund<sup>1</sup>, a project evaluating the quality of environmental journalism. Among others, these criteria were: "No Trivialisation / No Scaremongering", "Documentation / Evidence" (Presentation of studies / facts that elucidate the evidence), "Novelty" (The report indicates whether it deals with a new/newly discovered issue or whether this has been in existence for some time), and "Potential solutions and paths of action are presented".

Within the investigation period 15 articles focussing on microplastic in the marine environment were evaluated. Of those, only one met all 11 criteria, four reports met 8 to 10 criteria, eight fulfilled 5 to 7 criteria, and two articles met only 4 criteria. Accordingly, from the viewpoint of quality journalism only one third (5) of the articles examined can be regarded as excellent or good, indicating that the public is not adequately informed about the microplastic problem.

Rögener, W, Wormer, H. (2015) "Defining criteria for good environmental journalism and testing their applicability: An environmental news review as a first step to more evidence based environmental science reporting", *Public Understanding of Science* Volume: 26 issue: 4, page(s): 418-433

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Keywords: Microplastics, Marine Pollution, Marine Environment, German Newspapers, Quality Journalism, Quality Criteria, Mediendoktor Umwelt

## Compost - a potential source of microplastics: amount, source and composition of plastic materials in feedstock organic wastes

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Compost can be a potential source for microplastics, since organic wastes delivered as feedstock materials of compost may contain a significant amount of plastic contaminants like plastic bags and food packaging. Preliminary examination at a compost facility in Germany shows that plastic items account for 2.8 percent (by weight) of organic wastes collected for the production of



compost. Yet, the nature and extent of microplastics in compost are still largely unknown. To understand the links between feedstock, processing technology and the resulting product "compost", the plastic fraction in the feedstock has to be understood. As a part of our project "co(MP)ost" funded by Fritz und Margot Faudi-Stiftung, the seasonal variation in amounts, sources (items) and compositions of plastic materials in several compost- ing feedstock batches as processed by a composting plant in Darmstadt-Kranichstein are being investigated. The knowledge of the nature of the feedstock materials was gained through careful classification and sorting, giving an overview of the to-be-expected plastic contents in the com- post. Mostly plastic in the feedstock was of macroplastic size, often intact and easily discernible pieces among the municipal biodegradable waste. The first series of random sampling tests have showed polyethylene, polypropylene and styrene based polymers as the most common polymers. We hypothesise that the qualitative material composition of the output material will be roughly the same as the input material, albeit at smaller particle size. To validate our hypothesis, the nature and extent of plastic fractions in actual compost sam- ples are also to be analysed. Elaborate methods are to be developed to extract microplastics from compost as well as to determine the composition of the isolated microplastics by pyrolysis GC/ MS. Once the input and output material flows are known, single processing technologies can be evaluated for their plastic degeneration impact and therefore their microplastic generation potential.

Keywords: microplastics, compost, waste treatment, recycling, biodegradable

\*Speaker

## Comparison of one-time spiking, multiple spiking and dialysis tubing methods for the determination of plastic-water partition coefficient of one polycyclic hydrocarbons on microplastic

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Determination of sorption capacity of persistent organic pollutants (POPs) on microplastics (MPs) is essential to study ecological effect of POPs-loaded MPs in the aqueous environment. However, due to high octanol-water partition coefficients ( $K_{ow}$ ) as well as low water solubility, determination of sorption capacity of POPs on MPs in the laboratory is challenging. Here we present three methods to determine plastic-water partition coefficient of phenanthrene (Phen), one

of polycyclic aromatic hydrocarbons (PAHs), on low-density polyethylene (LDPE). Sorption tests were implemented in batch tests in the laboratory using conventional one-time and multiple spiking methods as well as a cellulose dialysis tubing method. The plastic samples tested here are LDPE pellets with a low amount of additives.

For the one-time spiking test, a high concentration of Phen in ethanol was spiked in a 2.5L glass bottle containing MilliQ-water and shaken. The well-mixed solution was placed into several smaller reactors and LDPE was added. For the multiple spiking test, batch reactors containing LDPE and MilliQ-water were prepared. A high concentration of Phen in ethanol was injected repeatedly into each batch reactor until the concentration became stable. For the dialysis tubing test, a closed dialysis tubing containing LDPE and MilliQ-water was placed in each batch reactor filled with MilliQ-water with Phen far above solubility. The water concentration outside of the tubing was expected to stay constant during the entire experiment. The concentration in the water phase for the first two tests as well as in the dialysis tubing for the third test was monitored using high performance liquid chromatography (HPLC). When the adsorption of Phen was completed, Phen on LDPE was to be extracted and quantified. Sorption capacity of Phen was derived from the experiments and methods were compared. PE-water partition coefficient of Phen were derived based on the sorption capacity using adsorption models.

Keywords: Partition coefficient, dialysis tube, spiking, polycyclic aromatic hydrocarbons, phenanthrene

\*Speaker

## Investigating tyre-road wear particles as contributors to microplastic waste

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The ubiquitous presence of microplastic waste within the marine environment has been well documented within contemporary scientific literature. Whilst the term frequently refers to debris such as microbeads, recent publications, which stem primarily from reports produced in Scandinavia, have indicated the wear generated from the abrasion of vehicle tyre tread against the road surface to be a potentially significant contributor to microplastic emissions. Their solid state, synthetic polymer base, insolubility in water, low degradation rate, and minute dimensions, allow them to be encompassed under this definition. These claims however are not supported by primary data, and the potential of these particles to reach recipient waters is uncertain. Additionally, tyre

road wear particles (TRWP's) do not respond well to the conventional methods of isolation and identification of microplastics. It is therefore not known if they are previously un-reported due to a low environmental presence, or an inadequacy of detection. Publications regarding the link between TRWP and microplastics have a general consensus that little or no reliable, or relevant quantitative data exists on their sources, pathways, and ultimate fate in the environment. Therefore there is a pressing need to establish the true scale of this issue. This study offers a better understanding of the characteristics of these particles, and proposes a protocol for the identification and quantification of TRWP's from environmental matrices. This study betters our understanding of the relative significance of these particles to microplastic waste, and indicates the likelihood of TRWP's reaching, and polluting the marine environment.

Keywords: micropalstics, marine pollution, tyre, road wear particles

\*Speaker

## Abundance and composition of floating marine macro litter on the eastern sector of the Mediterranean Sea

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Floating marine macro litter is known to be a widespread pollution problem, particularly, in the Mediterranean basin, but records on the abundance and composition of floating marine litter in the most eastern part are rare or unknown. Using a large observation platform (32 consecutive days, 137 transects, 1784 km) this study provides evidence of a high amount of floating marine debris in the Levantine sub-basin within the eastern Mediterranean, where small plastic debris accounted for > 90 % of the items surveyed. The values quantified in the present study tend to be higher than densities reported elsewhere in central and western Mediterranean, which may be related with several factors including circulation patterns and inputs from coastal sources. Significant correlations between floating macro litter density with wind force and sea state were found, strengthening the need to standardize protocols of visual surveys of floating macro litter.

Keywords: Marine litter Pollution, Plastics, Surface waters, Eastern Mediterranean

\*Speaker

# Polystyrene microplastics did not affect body growth and swimming activity in *Xenopus laevis* tadpoles

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Microplastics (MPs) reach the aquatic ecosystems by direct input or through the breakdown of larger plastic items. A growing number of studies has demonstrated that diverse MP typologies are widely distributed in the marine environment and can be ingested by diverse organisms, representing a serious environmental problem. Surprisingly, although the main sources of MPs for marine environments are inland surface waters, the information on the occurrence and the effects of MPs in freshwater ecosystems are still scant and often focused on invertebrate species or fish. Thus, the aim of the present work is to investigate the ingestion and possible adverse effects due to the exposure to polystyrene MPs ( $\text{Ø} = 3 \mu\text{m}$ ) on tadpoles of the Amphibian *Xenopus laevis*. Larvae at the developmental stages 36 and 37, prior to mouth opening, were exposed under semi-static conditions to 0.125, 1.25, and 12.5  $\mu\text{g/mL}$  of polystyrene MPs, and allowed to develop until stage 46. At the end of the exposure, the digestive tract and the gills from exposed and control tadpoles were microscopically examined, as well as changes in body growth and swimming activity. MPs were observed in the digestive system of samples from each concentration in a dose-dependent manner, but no adverse effects were detected; on the contrary, no MPs were found in the gills, even in samples exposed to the highest concentration. Tadpole growth and swimming activity were not affected by MPs. Our results demonstrated that polystyrene MPs can be ingested by tadpoles, but they did not alter *X. laevis* development and swimming behavior at least during early-life stages, also at high, unrealistic concentrations.

Keywords: polystyrene, *Xenopus laevis*, swimming activity

\*Speaker

## Importance of micro and nano-plastics characterization to assess biodegradation processes.

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The persistence of micro-plastics in the environment is a major concern that drive researchers to develop a wide range of new analytical methods to detect and quantify micro-particles. Under environmental constraints, the fragmentation of plastics ends with the production of nanoparticles that are even more difficult to monitor. These low molecular weight compounds can be eventually assimilated by microorganisms and their characterization is of major importance to understand the biodegradation processes. To meet this challenge, we developed a multi-disciplinary approach by combining analytical chemistry methods with physico-chemical and microbiology techniques. <sup>1</sup>H NMR spectroscopy, LC Orbitrap™ mass spectrometry, Dynamic Light Scattering analysis were used to characterize the size, the distribution and the chemical composition of nano-particles extracted from pre-aged polyethylene films. This methodology was applied before and after incubation with a bacterial strain to assess the specificity and the efficiency of biodegradation processes. The results give an extensive picture of the relative concentrations and the structural compositions of the extracted nano-particles. Oxidized micro-plastics release progressively nano-compounds with a molecular weight that does not exceed 850 Daltons. A significant part of these molecules remains stuck in the polymer matrix and can be released with a further oxidation. 95% of these overall nano-plastics were assimilated by a strain of *Rhodococcus rhodochrous* after 240 days of incubation. Interestingly, the larger molecular weight compounds completely disappeared from the culture medium while smaller ones were still detectable but at a very low level. This suggests the implication of extracellular enzymatic mechanisms leading to chain cleavages and opens new perspectives of research on biodegradation processes. This study highlights the relevance of multidisciplinary approaches to characterize nano-plastics and to better understand their biodegradation in the environment. It also underlines the importance to consider the nature of polymers, biological, chemical and physical parameters influencing the degradation.

Keywords: LC/MS, NMR, nano, plastics, biodegradation, multi disciplinary approach

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## Standardised Protocol for Monitoring Microplastics in Sediments.

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Marine anthropogenic litter, of which microplastics is part of, has long been recognised as an emerging pollutant of global concern with ubiquitous distribution and direct and indirect impacts on aquatic ecosystems, marine fauna and local economies. In order to contribute to the international debate on microplastics, we hereby propose a standardised protocol for monitoring microplastics in benthic and intertidal sediments from baseline data and literature review in international databases, as well as a definition for the term microplastics. The protocol developed under the scope of the JPI-Oceans BASEMAN project, is aimed at improving sampling, processing and increase the quality of microplastic data collection while also allowing comparison amongst different research studies. The protocol also takes into consideration different logistic and budget realities of EU member-states, providing all the relevant information for stakeholders to create or adapt their existing monitoring protocol to include the suggested recommendations on sampling, processing and identifying microplastics from sediment samples. This protocol can be used by a wide range of stakeholders ranging from research departments to state agencies in charge of monitoring programmes and it was designed to provide answers to research and/or monitoring question posed within EU legal frameworks such as the EC Marine Strategy Framework Directive (56/EC/2008), and particularly its descriptor 10 (Marine litter).

Keywords: marine litter, benthic, intertidal, policy, pollution

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## A comparison of new and conventional methods for isolating microplastics ingested by fish

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In recent years an increasing number of techniques have been developed to detect microplastics ingested by biota. In this study we propose a new simple and rapid method of microplastics extraction in comparison with methods commonly used. The new procedure consists of: fish gastrointestinal tract extraction; organic matter digestion with a mixture of HNO<sub>3</sub> 5% and H<sub>2</sub>O<sub>2</sub> 15%; vacuum pump filtration through a fiber glass membrane; microplastics identification under stereomicroscope.

Six types of polymers (nylon, polypropylene, polyethylene, polystyrene, polyvinylchloride, polyethylene terephthalate) were analyzed before and after the inclusion in different mixtures of

H<sub>2</sub>O<sub>2</sub> and HNO<sub>3</sub> in order to compare the potential plastic degradation among the tested solutions.

Changes in plastic items surface, colors and shape were evaluated with imaging software for microscopy (ZEN 2011 SP1) and processing software (ImageJ), before and after the treatments.

The Fourier Transformed Infra-Red Spectrometry analyses (FT-IR) were used to evaluate the integrity of polymers structure.

An homogenized pool, including gastro intestinal tissue and contents (from the esophagus to the cloacae), of 30 *Scomber japonicus* (Houttuyn, 1782), was subdivided in aliquots of around 9 grams each.

The proposed procedure for biological material removing (adding 10 ml of H<sub>2</sub>O<sub>2</sub> 15% and HNO<sub>3</sub> 5% solution for one gram of tissue at 40°C) was compared with two common methods, one using an oxidant agent as H<sub>2</sub>O<sub>2</sub> 15% at 50°C, the other a strong base as KOH 10% at 60°C .

Samples were processed using 10 aliquots for each protocol.

The results showed an efficiency over 95% on digested biological materials for the proposed procedure. Our results are comparable with those obtained using KOH protocol but with a clearer filtering membrane and an higher efficiency with respect to H<sub>2</sub>O<sub>2</sub> 15% alone.

Keywords: fish, marine litter, microplastic, method, ingestion

\*Speaker

## Plastic debris in *Posidonia oceanica* egagropiles in coastal waters

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Plastic is ubiquitous within the global marine environment, essentially becoming part of the environment and the ecology of these ecosystems. Several studies have documented direct or indirect ingestion of plastic in species due to confusion with preys or through filtering processes (Fossi et al., 2017). In addition, microplastics and plastics have already been identified floating in coastal areas (Compa et al., 2018). *Posidonia oceanica* is one of the most important species in coastal ecosystems of the Mediterranean Sea providing shelter and resources for other organisms. According to its live cycle, the root system and leaves shed away forming spheroids (egagropiles) of organic material which have the potential to retain other structures such as fish skeletons, sand and

other organic and inorganic materials. In this study, a total of 89 *Posidonia oceanica* egagropiles were collected floating at sea in two coastal areas of the Balearic Islands during the summer season 2017. Once at laboratory the perimeter of each egagropiles was measured and plastics inside them were visually sorted under stereomicroscope to identify whether marine plastic was becoming part of their internal structure. Preliminary results indicate that 48% of the egagropiles from the eastern coast of Mallorca (Cala Ratjada) contained plastics, while only 12% of the egagropiles from the northern coast (C'an Picafort) had plastics. In addition identified plastics have been validated with ATR-FTIR spectroscopy (Bruker HYPERION 2000) indicating that marine plastics with different polymeric composition are becoming part of these organic structures. Results from this study are important since it could be indicating a transference of plastics along different sea compartments.

Keywords: *Posidonia oceanica* egagropiles, Coastal ecosystem, Balearic Islands, FTIR

\*Speaker

## Sub-lethal and embryo-toxicological effects of chrysene-loaded microplastics on the Mediterranean mussel *Mytilus galloprovincialis*

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Plastic pellets and fragments resulting from the breakdown of larger objects are widely distributed in the marine environment and matter of great concern. Many reports indicate that microplastics (< 5mm in size; MP) may act as a vector of organic pollutants, including PCBs and PAHs, but less is known on the potentially associated effects on marine organisms. This study was conducted in the framework of the JPI-OCEANS project PLASTOX with the aim of investigating the effects of 3-um polystyrene MP loaded with chrysene, a potentially carcinogenic PAH, on different life stages of the Mediterranean mussel *Mytilus galloprovincialis*. Analysed endpoints encompass gamete fertilization, embryo-larval development and a battery of lysosomal (lysosomal membrane stability, neutral lipids), oxidative stress (lipofuscin content, catalase, glutathione S-transferase activity) and neurological (acetylcholinesterase) biomarkers analysed in adult mussels following a 7-day exposure. Preliminary exposure to chrysene (0.1 mg/L) did not affect gamete fertilization, while impaired morphological development of embryos. In adults, chrysene decreased lysosomal membrane stability and increased neutral lipids and lipofuscin content.



Moreover, chrysene increased glutathione S-transferase and reduced acetylcholinesterase activity in gill. The exposure to uncontaminated MP (20,000 particles/mL for embryos, 5,000 particles/L for adults) and the same concentration of chrysene-loaded MP (4 µg chrysene/mg MP according to GC-MS analysis) did not alter early life stages endpoints. Both MP alone and chrysene-loaded MP reduced the lysosomal membrane stability and increased the neutral lipid content. Only chrysene-loaded MP triggered an increased lipofuscin content, while a downregulation of catalase activity was induced by MP alone. No effect was induced by either chrysene-loaded MP or MP alone on the acetylcholinesterase and glutathione S-transferase activities. This study shows that chrysene-loaded MP altered lysosomal parameters and induced pro-oxidant effects. However, at our experimental conditions, chrysene-loaded MP did not induce effects greater than uncontaminated MP on embryos and adult mussels.

Keywords: Microplastics, Chrysene, mussels, biomarkers, early life stages

\*Speaker

## Marine litter, a threat to biodiversity conservation in the Santa Luzia Marine Reserve

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The marine litter that is brought out by the dominant currents (current of the Canarias) The Achados beach in Santa Luzia, accommodates 40% of the *Caretta caretta* nest of the Reserve and since 2010 the environmentalist association Biosfera 1, has been working hard, with the help of volunteers, to remove the waste from the area (mainly from fishing grounds, such as lines, nets, buckets, etc), otherwise it becomes a deadly trap for breeding individuals, but mainly for the young.

This year, 2018, the Biosphere is to identify some of the fishing waste to attempt to make the countries of origin of the same responsible. Because it is an uninhabited island, the cleaning campaigns have very high costs, which is intended to achieve, along with the main polluters, some annual budget to continue cleaning this important nesting area for marine turtles in the Reserve

Keywords: Marine litter, Environmental conservation, *Caretta caretta*, fishing waste, desert island, marine reserve

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## Praia limpa é a minha praia: An environmental project to reduce plastics and microplastics in marine and coastal environments

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”Praia limpa é a minha praia” (Beach clean is my beach) is a project of the Teacher Training College of the State University of Rio de Janeiro, Brazil; which aims to sensitize students from public and private schools of the different education segments, as well as the general public, regarding the presence of solid waste, mainly plastics and microplastics, in marine and coastal environments through environmental education activities. Using various tools such as lectures, storytelling, applications softwares developed by the project and working in both formal and informal spaces, the project has already reached about 5000 people. The increasing in public participation and invitations for partnerships and to carry out activities in the most diverse spaces show that the goal of raise awareness and create interest in the preservation of coastal environments has been achieved satisfactorily, showing that environmental education is one of the paths to the formation of critical individuals responsible for the environment where they live.

Keywords: Coastal environments, Education, solid waste

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## Preliminary study on microplastic assessment in the digestive system of marine mammals and turtles found stranded on Samos Island, Eastern Aegean Sea.

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The Eastern Aegean sea is characterized by the presence of a unique marine biodiversity: cetaceans, turtles and monk seals (*Monachus monachus*). To date, very few studies have been carried out for microplastic analysis in marine mammals and sea turtles even if studies of the digestive tract of different marine species have reported the presence of marine debris.

A preliminary analysis of the presence of microplastic was conducted on a total number of 40 animals of several ages and sexes found stranded on Samos island coastline during 2016 and 2018. Necropsies in situ or in the laboratory, when possible, were conducted for standard diagnosis analysis samples for the fresh carcass and the isolation of the digestive system for all the stranded animals. The methodology applied for microplastic analysis consists in collecting the entire digestive system from the first tract (oesophagus) to the last (last tract of the large intestine). All of the samples, after dissolving the organic matter, are filtrated. The filters are read using a microscope in order to identify and categorize the microplastics (sources, type, shape, colour and size). A "needle test" is conducted to distinguish between plastic pieces and organic matter (De Witte et al., 2014). A test for external contamination is conducted randomly. A high concentration and variety of microplastics were found throughout each tract of the digestive systems of all the animals analysed without any exception, confirming the wide pollution of plastic affecting the top predators of the trophic chain.

Keywords: marine mammal, Monk seal, turtle, Aegean sea, microplastic

\*Speaker

## Dynamics of floating marine debris in the northern Iberian waters: A model approach

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Floating marine debris is distributed worldwide through the oceans and poses a serious threat to marine ecosystems. Field data and model results show high concentrations of floating marine debris in the Bay of Biscay. In this work, the Regional Ocean Modelling System (ROMS), in conjunction with a particle-tracking model, has been used to study the distribution of floating debris in the northern Iberian waters. Long residence times were observed in the south-eastern Bay of Biscay, where the concentration of floating debris would be, on average, 2.1 times higher than in the north-western Iberian coastal waters, and 3.6 times higher when considering only the winter months. The analysis also suggests the existence of a seasonal influx of floating debris into the south-eastern Bay of Biscay, which would be greater during the winter, when an eastward transport of virtual drifters along the northern Iberian coast was observed. Both results - long residence time and influx of floating debris - support the hypothesis that the Bay of Biscay can be regarded as an accumulation zone of floating debris.

Keywords: Bay of Biscay, floating marine debris, marine litter, northwest Iberia, ROMS. \*Speaker

# The relevance of particle characterization for microplastic particles (MP) in the environment

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The main research questions (RQ) concerning MP include the understanding of: a) vertical and horizontal distribution in the aquatic environment and factors that affect the distribution, b) changes of material properties due to weathering and their consequences on the fate, behavior and transport of MP, c) MP toxicity and their dependence on different material properties. Depending on the RQ different relevant material properties have to be defined and determined with suitable particle characterization methods. While the distribution and transport of MP in the aquatic environment is mainly driven by properties like particle size, density and shape, the toxicity depends on the chemical composition, particle size, adsorbed pollutants and surface charge. The weathering of MP is linked to the polymer type, the embedded additives in the polymer structure, but also to the density. It determines the location of the polymer in the water column and thus which weathering processes can take place (with or without UV light). Our goal is to present a reliable set of parameters for each RQ. Therefore we are setting a variety of measurement techniques up that offer a broad characterization of MP within a limited time and resource effort. The particle size is important for several research questions, therefore we focus on its determination and combine dynamic image analysis with nanoparticle tracking to cover a broad particle size range. Both techniques allow the measurement of additional particle parameters like particle shape, concentration or surface charge. The surface charge of larger particles, fragments or fibers is analyzed by streaming potential measurements. For density measurements two techniques are used: helium pycnometry (dry samples) and flotation tests (wet samples). The link between RQ, measuring techniques and relevant material parameters will be presented.

Keywords: Microplastic particle analysis, streaming potential, size distribution, shape, concentration

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# Multimedia modeling of nano- and microplastics in the environment.

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Screening level multi-media models for the environmental assessment of nano- and microplastics (NMP) are not generally available. Here, we present SimpleBox4Plastics (SB4P) as the first model of this type, assess its validity, and evaluate it by performing rigorous sensitivity analysis. SB4P expresses NMP transport and concentrations in and across air, surface waters, soil, and sediment, accounting for particle-specific processes such as aggregation, settling and attachment, including those for nanoscale particles. The model solves simultaneous mass balance equations (MBE) using simple matrix algebra. The MBEs link all concentrations and transfer processes using first-order rate constants for all processes known to be relevant for NMPs. The first-order rate constants are obtained from the literature. The model accounts for emissions to air, soil, water and atmosphere such as those from direct sources, thermal cutting of polymers, 3D printing, and abrasion and wear of plastic items including car tyres. The output of SB4P is mass concentrations of NMPs as free particles, heteroaggregates with natural colloids, and larger natural particles in each compartment in time and at steady state (Figure 1). We evaluate the uncertainty in predicted environmental concentrations (PECs) by performing Monte Carlo (MC) simulations on the environmental fate, concentrations and speciation of NMP with sizes ranging from 100 nm and 10 mm. Realistic distributions of uncertainty and variability were applied for all of SB4P's input and model parameter values. We argue that this allows for the detection of the dominant processes driving fate and distribution of NMPs as a function of size and polymer type. Finally, SB4P predicted environmental concentrations are useful as background concentrations in environmental risk assessment.

Keywords: Multimedia modeling, Exposure, SimpleBox

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# Microplastics in the Belgian Part of the North Sea: Spatial variability and ingestion by benthic biota

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Global plastic production has reached 322 million tonnes per year in 2015. Due to careless waste management or accidental discharges around 10% of the newly produced plastics end up in the marine environment. Plastic debris in the oceans can be found in different forms, but in recent years especially microscopic particles known as microplastics (particles smaller than 5mm) are of growing concern. In 2015 the JPI Oceans project PLASTOX was established to investigate the ingestion, food-web transfer and ecotoxicological impact of microplastics on key European marine species and ecosystems. In the framework of PLASTOX benthic species of different trophic guilds (invertebrates, benthic-pelagic and demersal fish) were sampled in the Belgian Part of the North Sea. The organisms were digested using KOH (1M) after which the solution was filtered (20 µm). Suspicious particles were isolated from the filters and plastics analysed using infrared spectroscopy (µ-FTIR - ATR). Preliminary analysis from 4 stations that cover gradients in sediment and water column microparticle concentration show that microparticles are omnipresent with about 6-12 times higher concentrations in the sediment as compared to the water column. Microparticles were found in all of the 14 species that were analysed so far with black and blue fibers forming the majority of ingested particles. Concentrations varied between species (0.5-2.1 per individual) and were highest in benthic omnivores, while benthic-pelagic fish had less particles ingested than demersal fish species. Plastics formed the majority (96%) of particles in the sediment (77% Polyethylene terephthalate (PET), 22 % Polyethylene (PE), 1 % Nylon) and water column (80%; all PET), whereas only PET was found in the benthic biota that are analysed so far.

Keywords: microplastics, macrofaunal invertebrates, fish, continental shelf seabed

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# Fish, bivalves and polychaeta: microplastics ingestion in Portuguese Coastal waters

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Microplastics (MP) are ubiquitous in the marine environment and their presence can lead to harmful consequences to biota as they are easily ingested. This study provide a quantification of MP ingested by commercial fish, bivalves and polychaeta from Portuguese Coastal waters and aims to contribute to public awareness of the potential harm to human health. Two different fish species (*Trachurus trachurus*, n=82) and *Scomber colias*, n=82) were collected from Figueira da Foz and Sesimbra fishing ports and two different species of bivalves were collected in the Tagus estuary and in Porto Covo (*Mytilus galloprovincialis*, n=70; n=70) and in the Sado estuary (*Scrobicularia plana*, n=140). The polychaeta *Marphysa sanguinea* (n=30) used as fish bait was collected in Sado estuary and analysed for the first time regarding microplastics ingestion. MP fibres and fragments were the most frequent MP observed (79% comparing to 21% of fragments in fish and 83% comparing to 17% of fragments in bivalves). Fish collected from Figueira da Foz comprised 62% of the total MP detected and *S. colias* ingested 54% of the total MP. In total 67% of the fish and 28% of the bivalves were found to have ingested microplastics. The lugworm had the lowest percentage (17%) and mussels from Porto Covo had the highest (36%). Knowledge about the concentrations of MP in the water and sediment is needed in order to establish a possible relationship between the environmental conditions and animal feeding mode, as well as the relation between transfer of MP adsorbed contaminants (POPs), in the food chain and contribute to risk analysis for human health as these animals are frequently part of human diet.

Keywords: microplastics, ingestion, fish, mussels, fragments, fibers

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## Unravelling the pathogenic potential of microplastics in the Weser estuary (Germany)

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Microplastics (MP), as an emerging threat to the marine environment, have also recently been recognised as potential vector for the dispersal of microorganisms including pathogenic bacteria and/ or antibiotic-resistance genes (ARGs). Consequently, this poses the risk of propagating pathogens and ARGs into new environments in an invasive manner. While substantial research has already been conducted on MPs in the oceans, little is known about MPs in rivers and estuaries. However, rivers might serve as important transportation routes and as sources of MP, harmful microorganisms, and ARGs in the marine environment. This study investigates the occurrence of potentially human pathogenic bacteria in microbial biofilms in freshwater, estuarine and marine environments on MPs in contrast to wood as a natural substrate. As such, polyethylene (PE), wood, and tire wear particles were sequentially incubated in the German River Weser and the German Bight from freshwater- to marine conditions. Water samples and subsamples of particles were collected and transferred to selective media for the enrichment of potentially pathogenic coliforms (ESBL) and *Vibrio* spp. Obtained bacterial isolates were identified with Matrix-Assisted Laser Desorption/ Ionisation – Time Of Flight Mass Spectrometry (MALDI-TOF – MS).

Keywords: microplastics, pathogens, antibiotic, resistance, biofilm

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## Plastics in the beach sands of the Baltic Sea: Can some “background contamination” be defined?

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Plastics in general, and microplastics (MPs) in particular, are nowadays found on the beaches worldwide. The contamination pattern is however very patchy. This makes it difficult to differen-



tiate between the background contamination of the natural (marine) environment and by-chance pollution of the given locality due to touristic activity or proximity to litter point sources. Typically, larger litter objects are associated with urban areas, river mouths, etc., whilst MPs is dispersed everywhere. Whether there is a certain size of plastic particles, which can be taken as a border between the "background contamination" of the given marine environment, and the "littering of the particular beach" due to local human activity? To check this, a sampling was performed on sandy beaches of 100-km-long UNESCO National park of the Curonian Spit, located in-between Klaipeda (Lithuania) and Zelenogradsk (Russia) cities. The samples were collected on the beaches of the both cities and at 4 locations along the National park coasts in-between them. Larger beach litter (2 mm - 25 cm) was collected across the beach by the Sand Rake method (Haseler et al., 2018); sand samples for MPs (0.5 – 5 mm) were collected from the beach face, from wrack lines, and in-between the wrack lines, and analyzed in laboratory using the modified NOAA method (Esiukova, 2017). For active meteo- and hydrodynamic conditions of the Baltic Sea, the "background contamination" is associated with the MPs range.

Keywords: microplastics, beach litter, marine litter sources, beach monitoring, city areas, bathing beaches, human impact, Good environmental status

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## The Marine Plastic Microbiome: Microbial Colonization of Polymer Surfaces in the Arctic Marine Environment

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While sources and fates of plastic pollution are receiving growing attention, major knowledge gaps exist. Among these, microbial degradation (aka biodegradation) of plastics remains poorly investigated. The process of biodegradation begins with the formation of biofilm on the polymer surface; our study aimed to investigate microbial colonization of polymer surfaces in the Arctic marine environment around Tromsø, Norway. An immersion experiment was designed to assess microbiome community composition on four different types of pre-production microplastic (< 5mm in diameter) pellets (Low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS) and polyethylene-terephthalate (PET)) and rubber (a non-synthetic polymer used as a control) over a

period of 6 months at two different locations around Tromsø. Surface states of pre and post-immersion polymer samples were examined using Scanning Electron Microscopy. Samples were taken post-immersion at 3 and 6 months, and surface biofilm was subject to chemical and enzymatic digestion and DNA extraction by phenol-chloroform separation. Genotyping using 16S, 18S and ITS 2 rRNA gene amplification and next-generation sequencing on the Illumina platform was employed to identify bacterial, eukaryotic and fungal microbial life on the polymer surfaces. Investigation of the species richness and diversity within and among polymer types (alpha and beta-diversity, respectively) contribute key insights to the body of knowledge relating to the plastic microbiome and its potential role in polymer degradation. Taxonomic profiles were compared against a database of known polymer-degrading microbes to determine if any microbial degradation was likely under Arctic conditions. Several notable operational taxonomical units were identified including members belonging to obligate hydrocarbon-degrading bacterial species, marine fish pathogens, and members of families containing polymer-degrading bacterial species. Significant differences in community structure were noted between polyethylene-associated and free-floating fungal communities, as well as between PET-associated and both rubber and free-floating bacterial communities.

Keywords: biofilm, bioremediation, microbiome community, plastic pollution

\*Speaker

## Microplastics off Italian Coasts: A Comparison between Water Sampling Techniques

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Mediterranean Sea is a semi-enclosed basin, subject to intense anthropogenic pressures. Due to the very limited water exchange with Atlantic Ocean, Mediterranean potentially acts as a trap for microplastics (MPs). The area has been recognized as hot spot for plastic pollution, but different MP values has been reported which sometimes make spatial or temporal comparisons tricky. A number of reasons, including different sampling and lab techniques used, might explain discrepancies among studies. This work aims to compare two frequently used water sampling techniques: the Manta net, a 330 µm-mesh for collecting water surface samples and the Bongo net, a 200 µm-mesh for samples 60 cm-below surface. In July 2017, during the "Less Plastic, More Mediterranean" tour organized by Greenpeace, water samples were collected from 19 stations located nearby harbors and

Marine Protected Areas (MPAs) off Italian coasts, using both nets. In the lab, samples were analyzed for their plastic content and potential MPs were categorized according to shape, size and polymer type using Fourier-Transform Infrared (FT-IR). Differences in sample composition were assessed using Principal Component Analysis (PCA). The results highlight the need for unique and standardized procedures, otherwise we should always be careful when comparing and drawing conclusions. Overall, MPs were found in all the sampling stations, with remarkable values in areas highly populated and industrialized (e.g. Portici) but also in MPAs (e.g. Tremiti). This finding demonstrates the ubiquity of plastic pollution from which even MPAs, supposed to be pristine, are not immune.

Keywords: Mediterranean Sea, water sampling techniques, microplastic analysis, FTIR spectroscopy

\*Speaker

## Assessment of semi-persistent and emerging pollutants in microplastics on four Canary Islands beaches

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Microplastics represent a growing environmental concern for the oceans due to their capacity to adsorb chemical pollutants. The aim of this study was to monitor a wide suite of chemicals in stranded plastic (pellets and fragments) from different beaches of the Canary Islands. A total of 77 chemical substances were determined by gas chromatography-mass spectrometry. Thus, we quantify adsorbed persistent organic pollutants such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), as well as semi-persistent contaminants such as polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs). In addition, we included a panel of selected emerging contaminants such as chemical sunscreens (UVFs) and organophosphorous flame retardants (OPFRs). For this purpose, we collected samples from four beaches of three islands (Gran Canaria, Lanzarote and La Graciosa). All the selected beaches had a N or NE orientation attending to the prevalent marine current of this region. All samples showed contamination by several chemicals of each group of pollutants analysed and statistical differences were observed among beaches. In Figure 1 we show the general results considering the sums per group of pollutants. Thus,

the sum of PCBs and OCPs were significant higher in both beaches from Gran Canaria, the island most industrialized. In addition, the sum of UV filters was higher in those beaches more frequented (Famara and Las Canteras) than those beaches occasionally visited (Cuervitos) and remote visited (Lambra). A similar trend was also observed for sum of semi-persistent pollutants ( $\Sigma$ PAHs and  $\Sigma$ BDEs). In the case of BDEs, especially in preproduction resin pellets. Furthermore, the sum of OPFRs was significant higher in a city beach (Las Canteras), in both pellets and fragments, than the others beaches studied. Further investigation is necessary to understand the relationship between plastic types and adsorption for different pollutants.

Keywords: POLLUTANTS, PELLETS, FRAGMENTS, EMERGING POLLUTANTS

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## The retention time of microplastics in barnacle naupliar larvae

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Microplastic pollution is a growing global problem. High concentration of microplastic particles smaller than 50 $\mu$ m has been detected in Arctic sea ice, indicating that very small microplastics may be a serious problem in future. However, little is known about how long do these small microbeads will retain in marine larvae. In the present study, we evaluated the retention time of four sizes polystyrene microbeads (diameter 1.0, 6.8, 10, 20  $\mu$ m respectively) in four different species of barnacle naupliar larvae (The intertidal barnacles *Amphibalanus amphitrite* and *Fistulobalanus albicostatus* The turtle barnacle *Chelonibia testudinaria*, and the coral inhabiting barnacle *Darwiniella angularis*). Totally, there is a trend that smaller microplastics(1.0, 6.8  $\mu$ m) took longer retention time than larger microplastics (10, 20  $\mu$ m)t. Except for *A. Amphitrite* larvae which showed similar time to egest four different sizes microbeads. Despite the same trend, the duration to egest microplastics is quite different among different species. The retention time of *A. amphitrite* larvae is shortest, all of the four sizes microplastics can be egested within 90 minutes. *C. testudinaria* and *F. albicostatus* took longer, around 2 hours to egest microplastics. However, for coral inhabited barnacle *Darwiniella*, it took more than 5 hours to egest all the microplastics. This indicated that the impact of microplastics may be species dependent and we urgently need more studies to have a better understanding of the true effect of microplastic pollution.

Keywords: Retention time, planktonic larvae, barnacle naupliar larvae

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## Do polystyrene microplastic particles modulate the toxicity of pesticides? Mortality and biochemical responses of the snail *Marisa cornuarietis* and the non-biting midge *Chironomus riparius*

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Microplastics are of particular interest in ecotoxicology because they can interfere with organic substances like pesticides or pharmaceuticals, modulate their toxicity and in addition, mechanically affect exposed organisms. Whereas in the past most of the studies on microplastics have focused on the marine environment, there is still little knowledge about possible impacts on freshwater ecosystems. The aim of this study is to examine whether polystyrene (PS) microparticles (cryogenically milled granules, fractionated to < 100 µm) in combination with different organic pesticides influence health parameters in the giant ramshorn snail (*Marisa cornuarietis*) and larvae of the midge *Chironomus riparius*. Snails were exposed to PS particles (10,000 particles/L; < 100µm) alone and in combination with different concentrations of the molluscicide methiocarb (10; 50; 100; 300; 500; 1,000; 10,000 µg/L). As endpoints, oxidative stress (lipid peroxides, superoxide dismutase), proteotoxicity (stress protein level hsp70), neurotoxicity (inhibition of acetylcholinesterase) and mortality rate were studied. Up to now, no effects of PS particles on the investigated endpoints became obvious in the snails. However, not all data have been assessed so far. In a further study, larvae of *C. riparius* were exposed for 96 h to different concentrations of PS particles (150,000 and 1,000,000 particles/L; < 50 µm) alone and in combination with 1 µg/L of the insecticide thiacloprid (thia). As endpoints, mortality rate and acetylcholinesterase inhibition were investigated. The results show a significantly increased mortality in all treatments containing thiacloprid compared to control and microplastic-controls (Fig.1). This indicates, that PS particles do not modulate the lethal effect of thiacloprid. The analyses of acetylcholinesterase have not been finished yet, the results, however, will be shown on the poster.

The study is part of the joint research project MiWa ('microplastics in the water cycle') funded

by the German Federal Ministry of Education and Research (support code: 02WRS1378).

Keywords: organic pesticides, invertebrates, acetylcholinesterase, mortality

\*Speaker

## Pollution of Algerian coastal marine ecosystems by plastic waste

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## The Plastic-Seine research program: Integrated approach of microplastic fate and effects in the Seine estuary

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The Seine watershed (76,800 km<sup>2</sup>) can be considered as representative of river basins exposed to the impacts of intense human activity, leading to high pollution pressure. Chemical pollution is an additional threat to already very fragile aquatic ecosystems such as estuaries and coastal marine

areas. Few studies have focused on the contamination and impact of microplastics in estuarine ecosystems. The Plastic-Seine project (Flow and impacts of microplastics in the Seine estuary) involves six French laboratories associated in an innovative research project to study in an integrated approach, the occurrence and levels of contamination of the Seine Estuary by microplastics in all compartments of the ecosystem. Plastic-Seine also aims at evaluating their fate and their impacts on the food web at different levels of organization. Several field campaigns have been conducted since March 2017 at different sites and at different seasons, to sample surface water, sediments and seven representative species (fish, crustaceans, mollusks, annelids) of the Seine Estuary ecosystem. The first results indicate a moderate but general contamination of the different abiotic and biotic compartments by microplastics including fibers and fragments. Experiments in controlled conditions are underway to evaluate the fate and impact of environmental microplastic samples in three representative species of the Seine estuary. It appears that the microplastics are very quickly ingested but also eliminated (within a few hours) by the worm *Hediste diversicolor* and the common sole, *Solea solea* pre- and post metamorphosis larvae. This project should provide an initial assessment of the distribution and flow of land-based microplastics in the Seine Estuary and a first evaluation of the risk for aquatic wildlife. This project is funded by the Seine-Aval program and CPIER Vallée de Seine.

Keywords: estuary, microplastic, occurrence, water, sediment, biota, kinetic of transfer, toxic effects

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## Microplastics in Galway Bay: Preliminary results

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Microplastics, marine litter of anthropogenic origin, are a global problem with ubiquitous distribution in the marine environment. Due to their size, physical and chemical properties there are a multitude of ways in which the marine environment can interact with them. This study aims to establish a baseline for microplastics in Galway Bay in Ireland, by quantifying their densities in fauna, sediment and beam trawl samples from the benthos. A total of 30 sites were surveyed to collect benthic samples, including three beam trawls sites from previously trawled areas within the bay.

Preliminary beam trawl results show that although 100% of the species (n=24) surveyed contained microplastics, 15 of the 243 individuals surveyed did not contain any plastic in their guts.

No statistically significant differences were found between faunal feeding strategies with regards to microplastic consumption. Despite this, the predator feeding type, in the trawl, contained an average of 35 items versus 5 items in bottom feeders and 6 items in scavengers. Fauna species in sediments collected with a box corer, contained an average of 1 item per individual, except for parasitic species which had none. These results contrast with the average 3 microplastics per individual recovered in each of the sediment sample sites.

Microplastics from the beam trawl and benthic samples were mainly fibres (97%) followed by fragments (3%), with dominant colours being black and blue. Polymer types still need to be confirmed with  $\mu$ -FTIR analysis. It is expected that results may change with ongoing sample processing.

Preliminary results confirm ubiquity of microplastics in Galway Bay. This work will be enhanced with continued sampling/monitoring cruises over the next two years. Results will contribute to the creation of management tools that will be used to inform relevant stakeholders and to provide knowledge to the Marine Strategy Framework Directive, in Ireland.

Keywords: microplastics, monitoring, benthic, fauna

\*Speaker

## Snow as a route of microplastics from urban areas to sea

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In urban areas at northern latitudes, snow collected from roads, parking lots and market places is commonly dumped in seas or lakes nearby cities due to the lack of urban open spaces to store and melt the collected snow. For example in Helsinki, the capital of Finland, about one third of snow collected from urban areas is dumped directly into the Baltic Sea. However, this potential source of marine litter and microplastic pollution in aquatic environments has still remained unexplored. To our knowledge, this is the first study that examines the numerical quantity and the characteristics of microplastics in urban snow. In order to study waste and particularly microplastics in snow, samples were collected from three urban areas: a market place, a residential area and a road with heavy traffic. The waste particles in the melted and filtered ( $> 300 \mu\text{m}$ ) snow samples were visually identified utilizing the morphology of the particles, and the material of selected waste particles was analysed using a FTIR spectrometer.



On average, 16 600, 1 400 and 700 microplastic particles with size range of 0.3-4 mm were found per one cubic meter of melted snow collected from the residential area, the road side and the market place, respectively. These concentrations are considerably higher than the ones found in the effluent of the local wastewater treatment plant or in the sea water in Gulf of Finland. Road marking as well as polyethylene and polypropylene plastic particles were the most common microplastics in the urban snow samples. According to our results, the sources of the microplastics in urban snow include abrasion of asphalt pavement, road markings, car tyres, plastic products and textiles. This study shows that snow can be a remarkable route of microplastics to marine and freshwater environments in urban areas of northern latitudes.

Keywords: microplastic, route, snow, urban area

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## Assessment of the Trojan horse effect using PAH-contaminated polystyrene microplastics: sorption studies and responses of adult zebrafish

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The increasing presence of microplastics (< 5 mm, MPs) in the aquatic environment is being recognised as a global-scale problem. MPs are susceptible of being ingested by organisms and, depending on the size, of being internalised into the tissues, entering into the food web. Moreover, due to their hydrophobic nature and large specific surface, MPs can potentially adsorb other contaminants, especially hydrophobic organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), already present in the environment modulating their bioavailability and hazard. With the aim of assessing the potential role of MPs as carriers of pollutants, a phenomenon known as "Trojan horse effect", the sorption ability of polystyrene MPs (4.5 µm) for benzo(a)pyrene (BaP) and for a complex mixture of PAHs present in the water accommodated fraction (WAF) of a naphthenic crude oil was investigated. MPs were incubated in the PAH solutions for 24 h and, after centrifugation, PAHs were quantified in the supernatant by SPME/GC/MS. The percentage of

sorbed B(a)P from the total B(a)P solution was 21%. Sorption of the PAHs present in the WAF was closely related with the log Kow of each compound. Chrysene showed the highest sorption (87.55%) and fluorene the lowest (17.2%). Based on these results, an experiment with adult zebrafish was performed, where fish were exposed to MPs, to BaP-contaminated MPs, WAF-contaminated MPs, 5% WAF and ~ 0.1 µM BaP (21 µg.l<sup>-1</sup>) for 21 days. Preliminary results showed that zebrafish ingested 4.5 µm MPs, which were found abundantly in the lumen of the intestine. Further work is in progress to determine potential internalization of MPs into different organs, such as intestine, liver and gills, and the associated histopathological alterations caused, as well as transcriptomic effects.

Keywords: Microplastics, polycyclic aromatic hydrocarbons, sorption, aquatic toxicology

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## Microplastics in freshwater: surface water, sediments and benthos – a review.

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Microplastics in the environment are a novel contaminant, and therefore much research is still needed. Specifically, microplastics in surface water and sediment of freshwater systems, together with the interaction with biota, is a high priority research need. Here we present a comprehensive review of over 35 studies on microplastic occurrence in freshwater systems and effects on freshwater biota. Research on microplastic concentrations in freshwater and in freshwater sediment are limited and results vary by a factor of 10,000 for water and 100 for sediment. This appears to be due to different sampling-, pre-treatment- and analytical methods. Furthermore, little work has been carried out on the interaction between microplastics and freshwater biota, especially benthic macroinvertebrates. The capacity to take up microplastics has been documented in 12 studies; with only three studies documenting in situ ingestion. Studies on ingestion and effect of microplastics on benthic macroinvertebrates have thus far primarily been carried out under laboratory conditions, and often at very high concentrations. In addition, several ingestion studies have exclusively used beads, whereas the most typical microplastic morphologies found in freshwater are fibres and fragments. Furthermore, laboratory experiments have primarily analysed the ingestion and effect of a single polymer, whereas benthic macroinvertebrates will likely be exposed to a complex mix of polymers in environmental settings. In general, results are not consistent between studies and test species and more research is therefore urgently needed. Our

present study investigates the ingestion of microplastics by benthic freshwater macroinvertebrates. We will analyse MP content in a number of macroinvertebrates from different habitats covering a gradient of microplastic contamination. Thereby, we hope to assess their suitability as indicators in microplastic assessments by correlating the microplastic ingestion with microplastic in water and sediment.

Keywords: microplastics, freshwater, benthic macroinvertebrates, ingestion

\*Speaker

## Numerical tracking of debris from a shipping containers spill in the East coast of Australia

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Lost marine debris entails major environmental and navigation issues and leads to onerous clean-up work. Understanding and predicting the trajectories and fate of lost debris is crucial to minimise its impacts, and to reduce the cleaning efforts. On June 1st 2018, a total of eight- one containers were spilled from a Liberian-flagged ship, YM Efficiency, near Port Stephens (Southeast Australia). Debris was spotted to wash up along the coast of New South Wales, but most of containers have not been found to this day. In this study, we develop a regional numerical ocean model coupled to TrackMPD, a 3D non-Lagrangian model (Jalón-Rojas et al., MICRO 2018), to track and forecast the dispersion of this shipping containers spill. The model was applied to debris with different sinking behaviour in order to predict the fate of the diverse objects stored in the containers. So far, the modelled trajectories of floating debris accurately reproduce the beaching patterns of the spotted debris. Results show that both wind and the eddies of the East Australian Current highly affect the transport of the debris. The accuracy of the ongoing forecasting results is being evaluated at the same time than new debris are located.

Keywords: container spill, non, Lagrangian model, East Australian Current

\*Speaker

# Bioavailability of microplastic in laboratory exposure studies: What are we exposing to?

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To quantitatively study the uptake and effects of microplastic (MP) in planktonic species and other aquatic biota in a laboratory setup, it is paramount to monitor MP bioavailability. Many polymers are inherently hydrophobic and can elicit surface charges. Both factors can contribute to reduced bioavailability of MP in exposure media due to homoaggregation and adhesion to the surfaces of exposure vessels and analytical equipment. At present data on the stability and behavior of MP suspensions over time in laboratory exposure studies are lacking. Previous methods used to maintain MP of marginally negative buoyancy in suspension include mixing by water flow or air bubbling, ultrasound, overhead stirring and plankton wheels. Strong water flow and air bubbles are not typically used in exposures with planktonic species as they interfere with feeding behavior. In addition, air bubbling can cause heterogeneous distribution of MP in a suspension and ultrasound can damage the MP and change their properties. In the current study, the efficiency of overhead stirring to maintain spherical polystyrene (PS) MP (Ø10.4 µm) in suspension was assessed using flocculators. A coulter counter was used to measure MP suspension concentrations regularly during 24 h. We studied the impacts of exposure vessel material, PS bead start concentration, surface to volume ratio, stirring speed and the dispersant PVP40 (polyvinylpyrrolidone). All parameters tested influenced the number of PS beads in suspension, but none of them ensured a stable detectable PS concentration over time. Data from studies using plankton wheels indicate a similar issue. Overall, our findings indicate that MP increasingly attach to surfaces of exposure systems, sediment and/or surface over time, likely becoming gradually less bioavailable to test organisms. Consequently, we may be underestimating the uptake rates and effects of MP in aquatic biota to a variable and undefined degree.

Keywords: Polystyrene, adhesion, homoaggregation, bioavailability

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# Toxicity of microplastic fibres and their role as a source of emerging pollutants

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Microplastic fibres (MPFs) are increasingly being reported as one of the dominant forms of microplastic pollution in aquatic environments. Clothing and textiles produced from synthetic fibres such as polyester (PES), polyacrylic (PAC) and nylon (PA) are considered some of the main sources of MPFs. However, there has been little focus on the environmental fate and effects of MPFs compared to microplastic particles and fragments. Our previous findings show altered cell- and chlorophyll a production in marine microalgae exposed to microbeads, albeit at relatively high concentrations. In the current study, we investigate the potential for MPFs with different physical and chemical characteristics to elicit toxicological responses in freshwater and marine microalgae. Effects on algal production and photosynthetic activity were assessed as a function of polymer type, fibre length and concentration. In addition, the environmental fate of MPFs was studied by long-term mechanical and UV degradation studies using the same materials (PES, PAC and PA) under freshwater and marine conditions. A detailed chemical characterisation of the pristine test materials was conducted, using GC-MS, LC-MS and pyrolysis GC-MS to identify the type of additive chemicals present. Leaching studies were also conducted under the same freshwater and marine conditions to study the release of additive chemicals. The release of additive chemicals and their possible photodegradation to intermediate products was investigated as part of the UV degradation studies.

Keywords: microplastic fibres, aquatic environments, algae, production, photosynthesis, additive chemicals, photodegradation

\*Speaker

# Detection, characterization and removal of microplastics in wastewater treatment plants: Conventional vs innovative process

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Wastewater treatments plants (WWTP) are not specifically designed to retain microplastics (MPs), despite conventional processes can effectively remove most of these particles from the influent. Considering the high volumes treated daily, a considerable amount of microplastics can be still released through the effluents, highlighting the need to evaluate more efficient methods to reduce the input of MPs in aquatic ecosystems. In this study, the effectiveness in removing microplastics was assessed in a WWTP based on conventional activated sludge (CAS) processes and in a pilot scale AnMBR system made up of an upflow anaerobic sludge blanket (UASB) reactor coupled with an ultrafiltration unit of 0.03  $\mu\text{m}$  pore size and fed with the same wastewater treated by CAS. Microplastics were quantified in the liquid fraction collected at different steps of both the facilities, and characterized according to shape, size and polymer typology by microscopy and  $\mu\text{FT-IR}$  technology. The CAS process revealed an abatement efficiency of 82% with the greatest decrease of microplastics after primary and secondary settling: the influent contained 3.88 MPs/L (3880 MPs/m<sup>3</sup>), which were reduced to 2.12 MPs/L after primary settling, to 0.8 MPs/L after biological treatment and to 0.68 MPs/L in the effluent (680 MPs/m<sup>3</sup>). In comparison, the innovative AnMBR system removed the 97% of microplastics, providing 1.7 MPs/L after UASB reactor, further reduced to 0.1 MPs/L (100 MPs/m<sup>3</sup>) after ultrafiltration. MPs larger than 1mm were retained by intermediate steps of both systems. From the effluent of CAS process were predominantly extracted films and fragments of polyurethane and ethylene based polymers, in respect to the higher heterogeneity observed in the inflow. After the ultrafiltration unit only fibers, mainly of polyesters, were found. This study confirms the possibility to improve conventional WWTP with innovative technologies, such as ultrafiltration units, to limit microplastics' input in the environment.

\*Speaker

Keywords: Wastewater treatments plants (WWTP), Microplastics

## Impact of microplastics on phytoplankton

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Microplastic pollution is a growing global concern with potential impact on marine biota. Particles are ingested by marine organisms and can serve as a vector for transfer of contaminants with indirect consequences at higher trophic levels. The increase risk posed by the potential for biomagnification through the marine food web underpins the need to elucidate food chain effects, starting with phytoplankton – the base of the aquatic food web. We investigated growth and photosynthetic characteristic of a diatom (*Chaetoceros* sp.) when exposed to microplastics (green, polystyrene, 11-63  $\mu\text{m}$ ) with and without an adsorbed organic contaminant (phenanthrene). 30 mL of nutrient-rich synthetic seawater was spiked with 5 mg each either microplastic only or phenanthrene pre-sorbed microplastics. *Chaetoceros* sp. (15,000 cells mL<sup>-1</sup>) was then added, with a control as seawater only. The cultures were made in 3 replicates and maintained for 5 days with occasional shaking to avoid sedimentation under 6800 Lux light intensity with 12 hours lights on and off. Cell density of phytoplankton was measured using flow cytometry, while the photosynthetic characteristic was measured based on total chlorophyll (a, b, c) using UV-Vis spectrometry. Measurements were made daily for 5 days. Significant differences were observed at different exposure solutions for total chlorophyll and growth of *Chaetoceros*. The total chlorophyll after the 5-day culture was  $2.92 \times 10^{-3}$   $\mu\text{g L}^{-1}$  for the control,  $2.59 \times 10^{-3}$   $\mu\text{g L}^{-1}$  for microplastic only and  $1.97 \times 10^{-3}$   $\mu\text{g L}^{-1}$  for phenanthrene pre-sorbed microplastics. On the other hand, the cell density was 622090, 546630 and 207600 cells mL<sup>-1</sup> respectively. Increase in algal cells after 2 days of exposure was observed which 91830 cells mL<sup>-1</sup> for the control increased to 182780 cells mL<sup>-1</sup> for microplastics only and 94990 cells mL<sup>-1</sup> for phenanthrene pre-sorbed microplastics. This may be attributed to the response of algae when exposed to a particular stress.

Keywords: microplastic impact, phytoplankton, flow cytometry, chlorophyll, phenanthrene

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## The molecular effect of polystyrene microplastics in marine copepod *Tigriopus japonicus*

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Due to the indiscriminate use of plastics, the risk of contamination of the marine environment with plastics-derived microplastics is constantly being raised. Globally spread microplastics can affect all marine life, from microalgae to larger organisms. In this study, to understand microplastics-induced toxicity at the molecular level, we examined molecular responses to exposure to nano- (50 nm) and micro-sized (10µm) polystyrene microbeads in the marine copepod *Tigriopus japonicus*. MPs have accumulated in the intestinal track of the organism and in the female epippium. Several changes of antioxidant related genes were observed due to increase of intracellular reactive oxygen species (ROS), and the enzyme activity of antioxidant protein was significantly induced by micro - plastic exposure. The results of this study indicate that molecular level analysis can be used to understand the mechanisms of aquatic toxicity induced by microplastics in marine copepod.

Keywords: Polystyrene, Oxidative stress, ROS, gene expression, Enzyme activity

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## Bioavailability of benzo[a]pyrene transferred via microplastics to early life-stages of fish through experimental trophic food chains

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Because of their potentially high concentrations, very small microplastic particles (MPs) ingested by zooplankton organisms might pose a special threat to early life-stages of fish. The



present study describes exposure of fish larvae to MPs via trophic food chains from the very first days of feeding. Zebrafish (*Danio rerio*) and marine medaka (*Oryzias melastigma*) larvae were fed *Paramecium* spec. or *Artemia* spec. nauplii that had ingested 1 - 5  $\mu\text{m}$  (proprietary polymer by Cospherics) or 10 - 20  $\mu\text{m}$  fluorescent polyethylene particles. Hydrophobic organic pollutant uptake kinetics and potential accumulation patterns were analyzed with the transfer of fluorescent benzo(a)pyrene (BaP) from MPs to fish larvae and *cyp1a* expression analyses. Results indicate that *Paramecium* transferred very high amounts of only 1 - 5  $\mu\text{m}$  particles efficiently, whereas *Artemia* transferred both 1 - 5 and 10 - 20  $\mu\text{m}$  particles. Zebrafish and medaka larvae were feeding from the very first day of active food intake (2/3 days post hatching) on *Paramecium* with ingested MPs and from 6/7 days post hatching on *Artemia* nauplii with ingested MPs. Following MP ingestion, neither transfer to tissues nor accumulation of MPs could be detected with MPs egestion within hours following ingestion. Yet, BaP was efficiently transferred via trophic transfer, with higher exposure rates for small 1 - 5  $\mu\text{m}$  MPs when ingested via *Paramecium*. Induction of *cyp1a* expression revealed that BaP transferred from MPs was bioavailable in fish larvae. Given the dependence of the transfer rates from MP size and also re-desorption rates of chemicals depending of plastic type, very small microplastic and – most likely – nanoplastic particles might thus pose a significant threat for both early life-stages and juveniles of fish until adulthood with ongoing exposure via trophic transfer.

\*Speaker

Keywords: organic pollutants, trophic transfer, bioavailability, fish

## Identification and environmental evaluation of compounds migrated from polymers in marine medium biodegradation studies

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Polymers are made of monomers, but other substances are necessary for the polymerization reaction such as initiators, catalysts and solvents can also be used depending on the manufacturing process. In addition to this, the term plastic is referred to polymers to which additives have been added to give the desired properties for their processing or required final product characteristics. Examples of additives include pigments, light stabilizers, flame retardants, plasticizers, antimicrobials and antioxidants among others. All these non-polymeric components are usually of

low molecular weight and, therefore, can migrate from the plastic product to air, water and other contact media. On the other hand, the polymerization reactions are rarely complete and, therefore, unreacted monomers can also be found in the polymeric material. The type of substances that are formed during the abiotic and/or biotic degradation of polymers varies according to the type of polymer, the degradation mechanisms, the presence of polymerization impurities and the environmental effects. Some of the raw materials, intermediates or by-products of the polymers can sometimes be more toxic than the monomer itself, therefore, their environmental impact must be evaluated. Respectful additives with the environment should be selected to diminish their impact when plastics undesirably reach the ecosystem. The work presented herein is focused in the environmental impact of these compounds in the marine medium, as the accumulation of microplastics in the sea is of high environmental concern. The migration to marine medium of conventional and alternative additives added to polyamide (PA), low density polyethylene (LDPE), polyvinyl chloride (PVC) and polyhydroxy butyrate (PHB) have been evaluated during biodegradation studies in laboratory scale reactors. Biodegradation of the polymers and the formation of intermediate compounds have been determined for the subsequent study of their toxicological effect on sensitive marine organisms.

Keywords: Microplastics, environmental impact, migration, polymer additives, biodegradation

\*Speaker

## Review of existing monitoring programs for marine litter in the Mediterranean Sea

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The Marine Strategy Framework Directive (MSFD) is a European policy instrument that provides a framework in which Member States (MSs) must take the necessary measures to achieve or maintain the Good Environmental Status (GES) in their own EU's marine waters by 2020. GES is assessed by 11 descriptors, among them Descriptor 10 (D10) addresses Marine litter defining "Properties and quantities of marine litter do not cause harm to the coastal and marine environment". Moreover, the MSFD has a specific objective which is to further improve monitoring programs emphasizing the need to establish appropriate coordinated monitoring at (sub-)region level. Considering the different indicators for D10 (beach litter 10.1.1, floating litter 10.1.2, micro-litter 10.1.3 and litter in biota 10.2.1) a review of existing monitoring in the UE Mediterranean MSs has been realized. This analysis provided detailed information on gaps, commonalities and differences amongst EU MSs monitoring programs. Methods used to estimate abundance,

distribution and composition of marine litter (macro and micro) on sea compartments (e.g. sea floor, sea column and sea surface) and biota showed high variability across MSs. There are countries with few detailed methodologies for one indicator, such as France and Spain, which present several subprograms for all the indicators, and others with none, like Croatia for indicator 10.1.1, and Cyprus and Malta for 10.1.3. Special attention has been carried out to compare temporal and spatial resolution of monitoring programs as well as the parameters measured. It is remarkable that the subprograms established to analyze the micro-litter indicator are poorly defined. Considering the MSs monitoring in each (sub-)region in accordance with UNEP MAP plans, the present study proposes tools to harmonize and standardizes monitoring across MSs

Keywords: Marine Litter, MSFD monitoring, Mediterranean Sea, GES

\*Speaker

## Is *Mullus barbatus* a good bioindicator of microplastic marine pollution? Spatial and temporal comparison of microplastic occurrence in the NW Mediterranean.

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The Mediterranean Sea is a marine area with a high incidence of contamination by plastics as it is a closed basin surrounded by remarkable populated countries. Currently, microplastics have been focused much attention, especially on quantifying them in different areas and studying their potential vehiculization to other pollutants. The ingestion of this type of marine litter has also been described in many fish species, however, the potential impact that microplastics can have on marine organisms in wild remains totally unknown. Thus, the possibility that they can reach the trophic chain maintains an open discussion of the possible implications in marine communities at ecological level and human health. Red mullet (*Mullus barbatus*) is a fish species commonly used as a bioindicator for a large amount of pollutants and it has been also reported to ingest microplastics. In our current study, we aim to determine the potential impact of microplastics in *Mullus barbatus* using a refined methodology. A multidisciplinary approach (integrating environmental variables, microplastic quantification, fish condition, histopathological and parasitological assessments) have been carried out in order to infer the possible effects of plastics in the red mullet fitness. We aim to elucidate if this species could be used as good bioindicator of microplastics pollution. This study

was developed in both temporal and spatial scale: (1) we analyze if the amount of microplastics has increased during the last decade (11 years) and its potential impact in red mullet samples; (2) we also quantified the presence, amount, and potential impact of microplastics in red mullets obtained at two sampling stations in the NW Mediterranean, comparing a low impacted zone to another with a higher anthropic impact. Details, results and conclusions will be reported during the congress.

Keywords: Microplastic ingestion, *Mullus barbatus*, Bioindicator

\*Speaker

## Microplastic retention in a Danish storm water pond

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*Microplastic retention in a Danish storm water pond Kristina B. Olesen, Diana A. Stephansen, Nikki van Alst, Jes Vollertsen, Department of Civil Engineering, Aalborg University, Denmark.*

Large amounts of microplastic enter our environment with runoff from urban areas. Stormwater ponds are known to retain a wide range of micropollutants. However, no study has, to our knowledge, investigated the microplastic retention efficiency of a stormwater pond. In this study we present results from measurement of microplastic in stormwater from a retention pond, as well as sediments and fish from the same pond. We find low but not insignificant concentrations in both the water phase (4 µg/L) and pond sediment (400 µg/g). The results indicate (based on a rough estimation) a retention efficiency of 95 % (see figure 1). Hence the microplastics that enter the pond are accumulating in the sediments, making it a possible hotspot.

Microplastics are an inhomogeneous group of particles consisting of many different materials. It is hence rather challenging to measure it in natural systems, especially those that contain much organic material other than plastics. In this study, we therefore put focus on not only the measured microplastic concentrations, but also the methods and protocols to determine the plastic particles. We show how methods are optimized to micro FT-IR imaging, and how reliable the quantification methods are. The target particles of this study are in the size range 10 - 500 µm. The data obtained by FPA-µFT-IR-Imaging are processed by MPhunter – a dedicated software for automated MP detection. Figure 1. The sampling location and the microplastic fingerprint in the water and the sediments. The fingerprint from the water shows a larger variation in detected plastic types compared to the sediment. This could indicate that some plastics are retained to a lesser degree by the pond.

Keywords: Stormwater pond, runoff, micro, FT, IR Imaging, sediment, Fauna. \*Speaker

# Pilot study on possible micro plastic occurrence in marine mammals from German waters

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In the last years, especially the visible fractions of macro- and meso-scaled plastics were shown to be of concern, but the presence and effects of microplastic particles (MPPs) came more into focus of modern research. For instance, 30 sperm whales stranded in 2016 in the North Sea, of which nine individuals showed ingestions of macro plastic, e.g. fishing net or a car part (Unger et al 2016). In contrast to macro plastic, knowledge on MPPs, especially its accumulation in and physical influence on marine top predators is still insufficient.

We analysed faeces samples of three marine mammal species inhabiting German waters (harbour porpoise, harbour and grey seals) in order to gain information on micro plastic occurrence. Since the 1990s, the Institute for Terrestrial and Aquatic Wildlife Research (ITAW) regularly conducts necropsies of marine mammals found on the coasts of Schleswig-Holstein, Germany. Faeces sample collection started in 2014 with removing parts of the intestine with faeces content during necropsies according to an established protocol to avoid secondary pollution and conservable loss. With those valuable data collection, we were able to analyse the occurrence of MPPs in faeces samples in marine mammals from German waters for the first time. After isolation, the MPPs will be verified and identified if possible with the help of Fourier-Transform-Infrared spectroscopy (FT-IR). The investigation of marine debris of all sizes and its impacts on marine biota and habitats are essential for implementing the European Marine Strategy Framework Directive (MSFD). This includes the ubiquitous MPPs and its occurrence and transport in the marine food web. Especially, marine mammals as top predators are predestinated to accumulate both, MPPs and their associated chemicals.

Keywords: Micro plastics, occurrence, faeces samples, harbour porpoise, harbour seal, grey seal, North Sea, Baltic Sea

\*Speaker

# Biodegradability of pristine and weathered car tire particles and their individual components

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The wear of car tires is a contributor to particulate matter pollution, chemical pollution, and microplastics pollution. Tire wear particles are generated from the interaction between tire tread and road surface and released into the environment. The tire tread consists of a polymeric blend of natural rubber (NR), isoprene rubber (IR), butadiene rubber (BR), and styrenebutadiene rubber (SBR), compounded with carbon black or silica as a filler, along with extender oils, and additives responsible for vulcanization and protection. Biodegradability of car tire rubber was investigated following the OECD 301F guideline and using OxiTop instrumentation to determine complete mineralization under aerobic conditions. Pristine and UV-weathered tire particles were tested using both activated sludge and soil as inoculum. Effects of particles size (< 63 µm, 63-90 µm, or 90-125 µm) and inoculation time (4 or 11 weeks) was investigated. To assess biodegradability of individual components the following materials were tested: devulcanized tread particles (DTP), NR, IR, BR and TDAE oils.

Pristine and weathered rubber exhibited low but measurable biodegradation levels in the presence of activated sludge (3.8-7.6% ThOD) and soil supernatant (0.8%-2.3%). No relation between particle size and biodegradability could be established, likely due to similarity in sizes. Extending the inoculation time from 4 to 11 weeks increased the biodegradability from 6.7% to 9. No improved biodegradability was found when comparing DTP (3.46%) to tire particles (4.13%). Of the individual substrates, NR showed the highest degree of biodegradation (34.32%), followed by TDAE (7.13%). BR and IR showed no or negligible biodegradation. Overall, ready biodegradability tests proved suitable to obtain information on degradation of car tire rubber. The findings of this study provide a starting point for understanding the environmental fate of tire wear particles, providing an insight into the degradability of the tire wear rubber as well as its individual components.

Keywords: car tires, tyres, road wear, degradability, environmental fate

\*Corresponding author: fabp@env.dtu.dk †Speaker

## Have your say: What are the risks of microplastics?

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Up to date, our knowledge on the impacts of microplastics on human and environmental health is incomplete. Nevertheless, opposing perceptions of the risk of microplastics divide the scientific community: Whereas some believe microplastics are or can become a threat for our ecosystems in the near future, others argue that they pose no risk due to their low environmental concentrations. Whether or not science considers microplastics as issue, societies have already decided to take action on this environmental issue. A range of policies aims at reducing microplastic entry in water bodies (e.g. ban of microbeads). These actions are legitimated based on public rather than on scientific consensus and are implemented in the absence of a comprehensive ecotoxicological risk assessment. In that regard, we want to ask the community of microplastics researchers attending the MICRO2018 for their opinion. Hereby, we hope to broaden and strengthen the scientific perspective on the environmental risk of microplastics. On the basis of an interactive poster, we want to I) obtain expert opinions whether and why they perceive microplastics as a risk, II) reflect on the role of ecotoxicology in this complex issue and III) collect ideas on how to conceptualize microplastics for the purpose of environmental risk assessment taking into account their physico-chemical heterogeneity (no single chemical but a diverse and complex composition of different polymers, additives, sorbed chemicals and organisms as well as of different sizes and shapes).

Keywords: microplastics, risk perception, environmental risk assessment

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## Adsorption of natural organic matter on microplastic and its effect on dispersion stability

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While the sources and sinks of microplastic (MP) are currently well-addressed, the environmental behaviour of MP particles remains unpredictable. Rivers are transport vectors from sources (places of human activity) to sinks (lakes or oceans) with a lot of uncertainties involved in the transport process. A large discrepancy between lab work and environmental findings on MP

particles is the interaction with water constituents acting as dispersants. Virgin polymers are highly hydrophobic leading to homo-agglomeration and attachment on glassware surfaces whereas environmental MP is stably dispersed in water. Particle density is also altered: plastics of originally low densities such as polypropylene and polyethylene are found in sediments whereas those with high densities such as polyvinylchloride and polyester are also reported to be found in the water column, e.g. in manta trawls (Sighicelli et al. 2018). This indicates a transformation of physical properties, leading to different behaviours in the environment as compared to in the lab. Interactions of MP with natural organic matter (NOM) were shown to influence the stability of MP in dispersions (Chen et al. 2018) and are likely the most important factors for behavioural changes. Therefore, sorption capacities of different MP particles (polystyrene, polyethylene, acrylic glass, etc.) for NOM fractions were analysed. MP particles were produced by cryogenic milling. Particle sizes and dispersion concentrations were determined by light extinction particle counting. Changes in the zeta potential and agglomeration behaviour were examined in regard to dispersion stability. Both pH and ionic strength of the media were systematically varied to identify their respective impacts. PS was loaded with up to 0.78 mg DOC/g depending on NOM type. Furthermore, NOM sorption decreased homo-agglomeration of particles and had a bivalent effect on the zeta potential. The results also indicate a stabilizing effect of adsorbed NOM, explaining the dispersibility of MP in natural waters.

Keywords: dispersion, agglomeration, NOM sorption, transport behaviour, zeta potential

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## Microplastic at Slovenian beaches: Study repeated

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Plastic litter is gaining attention in environmental science, public perception, and, finally, in politics. Macro- and microplastic pollution of rivers, oceans, and beaches are regularly reported. However, to our knowledge nobody studied the temporal dynamics of plastic pollution at beaches. This study is a re-investigation of a former work done in 2012 on the occurrence, distribution and composition of microplastic at Slovenian beaches. Similar methods in field sampling and particle isolation were applied. The study was extended by including additional beaches, addressing seasonal aspects (winter and summer) and introducing quality assurance/quality control checks. Furthermore, the chemical composition of isolated plastic items was verified by ATR-FTIR spectroscopy. Only about 10 % of the putative microplastic items were decisively recognised as



synthetic material. Secondary microplastics were present as fragments, fibers, films, and foams. Primary microplastics were absent at all. The microplastic concentrations of the present study were distinctly lower than in the former study. The low pollution of Slovenian beaches could be the result of regular beach clean-ups of touristic beaches, or an improved environmental consciousness of citizens and tourists. This study emphasizes the importance of continuous microplastic monitoring to understand the annual microplastic contamination and its' seasonal and inter-annual dynamics.

Keywords: Adriatic Sea, Sediment Samples, Density Separation with NaCl

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## Do microplastics induce oxidative stress in marine invertebrates?

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The global production of plastics increased 20-fold in the last five decades. Simultaneously, environmental pollution by plastic litter became a rising issue. Marine plastic litter can have adverse effects on marine vertebrates and invertebrates. Upon ingestion, smallest plastic fragments may enter organs and even penetrate into cells where they may cause imbalances of the cells' redox state. The occurrence of oxidative stress can have adverse effects on cell membranes, proteins, or DNA. Here we studied the ingestion of microplastics by marine invertebrates, the possible transfer into cells of the digestive tract, and the cellular effects in the midgut gland. The Atlantic ditch shrimp (*Palaemon varians*) was chosen as model species. It inhabits coastal regions, estuaries, and brackish water systems, which are strongly exposed to anthropogenic pollution. Fluorogenic polystyrene microbeads were offered with the food and served as tracer. Uptake into the digestive organs and, particularly, into the midgut gland was analysed by fluorescent microscopy of cryostat sections of the digestive tract. An increase in the cellular defence mechanism against oxidative stress was verified by the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Formation of reactive oxygen species (ROS) indicated NADPH oxidase activity, a superoxide (O<sub>2</sub><sup>-</sup>) catalysing enzyme. The expression of NADPH oxidase in *P. varians* was verified by PCR-amplification. The outcome of this work may help identifying cellular reactions after exposure to microplastics and indicate a toxicological impact on cells and whole organisms.

Keywords: Crustaceaens, Histology, NADPH oxidase, Enzyme activity, RNA

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# Combined effects of microplastics and gold nanoparticles on the marine microalgae *Tetraselmis chuii*

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Microplastics are global pollutants that have been found to modulate the toxicity of other pollutants. Nanoparticles have been increasingly used and they are suspected of being important environmental contaminants in anthropogenic impacted areas but the current situation is largely unknown. The potential influence of microplastics in the toxicity of gold nanoparticles to the marine microalgae *Tetraselmis chuii* was investigated. *T. chuii* cultures were exposed for 96 h to ~ 5 nm diameter gold nanoparticles (AuNP) and to virgin 1-5 µm diameter microplastics (MP), alone and in mixtures. The effect criterion was the inhibition of the average specific growth rate. AuNP alone and MP alone did not cause significant ( $p > 0.05$ ) decrease of *T. chuii* average specific growth rate up to the low ppm range. The mixture containing the highest concentrations of both substances significantly ( $p \leq 0.05$ ) reduced the average specific growth rate of the microalgae. Therefore, the mixture was more toxic to *T. chuii* than its components individually, indicating that the presence of MP in the water increased the toxicity of AuNP to *T. chuii*.

Keywords: Microplastics, Gold nanoparticles, *Tetraselmis chuii*, marine ecosystems, microalgae, mixtures

\*Speaker

# May polyethylene microplastics affect ephyrae jellyfish survival and behaviour?

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The aim of this study was to verify whether polyethylene microplastics (MPs) could affect the survival and behavior of *Aurelia* sp. jellyfish, a new and sensitive model recently proposed for ecotoxicological surveys. Ephyrae were exposed to environmental and high (0.01-10 mg/L) concentrations of 1-4 µm and 4-6 µm MPs. After 24 and 48 hours, acute (percentage of im-

mobility) and behavioural (percentage of frequency of pulsation) endpoints and MP build up were investigated. MPs significantly affected ephyrae survival and behavior at all concentrations after 24 h of exposure, independently from MP size. In addition, it was possible to quantify the toxic effect by means of EC50s. MPs were observed on gelatinous body surface and mouth, likely attached on the surface, rather than ingested. These findings were related to a mechanical disturbance due to MPs, since contaminated ephyrae exposed to clean seawater showed a full recovery after 48 h. In conclusion, short-term exposure to MPs induces a toxic effect on both survival and behavior in a zooplankton species: independently from the size, polyethylene MPs temporarily affect both immobility and frequency of pulsations. The comparison of these results with those available in literature highlights how *Aurelia* sp. ephyrae are more sensitive than other marine invertebrates used so far for MP toxicity assessment.

Keywords: Ephyrae, Jellyfish, polyethylene microplastics, Behaviour, Recovery

\*Speaker

## Biological Effects of virgin and weathered Microplastic Particles and their Leachates

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Studies on the potential effects of microplastic (MP) particles in the aquatic environment are numerous. However, many laboratory studies apply pristine particles, which may be of limited environmental relevance given that factors such as UV light irradiation, mechanical stress, salinity and biofilm growth can influence the weathering of plastic debris. The aim of this study within the JPI Oceans-funded project WEATHER-MIC was to assess the impacts that weathering has on the effects of MP particles and their leachates on organisms and microbial communities. This poster summarizes our recent work on potential effects: (1.) Impact of MP particles on organisms: exposure to different fractions of virgin and weathered MP under controlled conditions. From the observation of apical endpoints in the acute toxicity assays, concentration-response relationships for the different fractions were deduced. A critical evaluation of the suitability of the applied test protocols for the assessment of adverse effects of MP was performed.

(2.) Influence of aging plastic on biofilm structure, function and community tolerance: Natural biofilms were grown in microcosms on different types of aged and pristine polymeric substrates. To study the influence of weathering on the attachment and succession of biofilms, sum parameters such as biomass, pigment profiles, cell counts and sequencing data were studied. (3.) Mixture effects of chemicals liberated from the most common polymers during weathering toward daphnia and algae. Plastic degradation products and additives were liberated during simulated weathering by intense UV light in artificial seawater. The chemicals were enriched by solid-phase extraction or extracted from pristine particles by ultrasonic-assisted extraction using solvents. The concentrated leachates and solvent extracts were then dosed into bioassays. The results may help to understand biological effects of chemicals that may leach out of weathering plastic material..

Keywords: weathering, organismic toxicity testing, plastic, aquatic environment

\*Speaker

## Microplastics in benthic, pelagic and demersal species along the North Sea coastline of Schleswig-Holstein, Germany

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The aim of the study is to evaluate the occurrence and distribution of potential microplastic contamination in benthic, pelagic and demersal species within the investigation area along the eastern German North Sea coast and to assess the suitability of different species for future monitoring purposes. Sampling of species took place in September 2017 and May 2018 at four different sites along the North Sea coastline of Schleswig-Holstein, Germany. Species were sampled with 20 individuals per sampling period and comprised the benthic species blue mussel (*Mytilus edulis*), common periwinkle (*Littorina littorea*), common cockle (*Cerastoderma edule*), soft-shell clams (*Mya arenaria*), pacific oyster (*Magallana gigas*), lugworm (*Arenicola marina*), and green crab (*Carcinus maenas*). Additionally, the pelagic atlantic herring (*Clupea harengus*) and the demersal species common shrimp (*Crangon crangon*), European plaice (*Pleuronectes platessa*), common sole (*Solea solea*) and viviparous eelpout (*Zoarces viviparus*) were sampled. Concerning the benthic species both residues after 24 hours of incubation in filtered saltwater and subsequently the whole organism were investigated. Concerning fish, stomach and guts were dissected and further

analyzed. Laboratory analysis comprised digestion with KOH/NaClO and density separation using NaI. Samples were transferred to cellulose filters and were stained with the lipophilic dye Nile Red prior to counting and scaling of microplastic particles under a fluorescence microscope (AxioLab A.1, Zeiss). A subsample was additionally analyzed with micro-Raman imaging (DXR2xi Raman Imaging Microscope, Thermo Fisher Scientific). Results on microplastic findings both for the investigated species and potential spatial patterns will be presented.

Keywords: microplastics, benthic species, pelagic species

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## Microplastics in the Marine Environment and Biota of Israeli Coastal Waters

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Microplastics are being recognized as a major marine pollutant throughout the oceans of the world. While research has shown that a wide range of marine biota are consuming microplastics, mainly through assessing target indicator species, little research has been done evaluating multiple fish species of different trophic levels and ecological niches in a single system. The present study aims to understand the extent to which microplastics have entered the marine food web in Israeli Mediterranean waters. The following set of measurements is being used; 1) screening for microplastics in marine fish of Israeli waters, 2) assessing the differences in microplastic mass compared to plankton mass in surface waters, and 3) screening of microplastics in bivalves to identify potential bioindicators. In this study 793 fish from 36 different species were collected using commercial trawling and a manta trawl simultaneously. Additionally, 10 individuals of the bivalve *Spondylus spinosus* were collected from five different sites along the Israeli coast known to have different levels of anthropogenic activity. Preliminary results show that of 106 fish specimen (9 species) assessed thus far, 24 have some type of microplastic contamination in their gastrointestinal tract. Except for a single blue film found in the predatory reef-associated *Alepes djedaba*, all microplastics observed are microfibers. Confirmation of the chemical makeup are awaiting microscopic-FTIR analysis. Manta trawl contents collected from fish collection site presented relatively high quantities of microplastics to that of plankton. However, this data is still being

processed. As no research has been done on microplastics in the biota of Israeli waters, this research will provide a baseline study for local waters. Additionally, the approach of this study allows for focus to be removed from the individual and, instead, looks at an entire population, thus contributing insight to microplastic behavior within a trophic system.

Keywords: fibers, trophic system, marine, fish contamination, plankton, bivalve, bioindicator, biota

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## Uptake of differently sized microplastics in gut passage by different species of *Daphnia*

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Microplastics are synthetic polymers with a diameter smaller than 5 mm and down to the nanoscale, have a widespread occurrence and negative effects on different trophic levels have been described. The freshwater crustaceans *Daphnia* forms part of the plankton community making them an important indicator species in the foodchain. The *Daphnia* family includes species ranging in size from *D. magna* (2.3-5.0 mm) to *D. ambigua* ( $0.88 \pm 0.01$ mm) which span a similar range of sizes as micro and nanoplastics, thus suggesting that different members of this family may be differentially sensitive to or affected by different sizes of micro or nanoplastics. This work presents a first analysis of the effect of *Daphnia* body and gut size on uptake of microplastics of different sizes. We investigated the ingestion and effects of polybead carboxylate microspheres (0.1, 1.0 and 10  $\mu$ m) on freshwater cladocera of different body sizes after 24 to 72 hours exposure to a range of mass concentrations. The hypothesis tested was that the size of microplastic particles preferentially taken up by the organisms will scale with organism size, due to differences in their gut sizes and filter feeding capabilities. We assessed the uptake, accumulation, and depuration of the microplastics in *Daphnia* species using stereomicroscope measurements. Image analysis approaches were utilized to calculate gut area in an effort to quantify particle uptake. Rapid accumulation in the gut was observed after exposure to all particle sizes in *D. magna*, with the smaller particle sizes being detectable in the guts of neonates of all three species and are presented here for the first time.

Keywords: *Daphnia*, cladocera, microplastics, freshwater systems

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## Exposure to microplastic: ingestion/egestion rates and behavioural impact on *Solea solea*

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Microplastic (MP) occurrence is ubiquitous in fresh and marine waters and species living in Estuary are particularly exposed to this kind of pollution. In this context, the Seine River is interesting for investigating the impacts of MP on fish, because of intense and numerous human activities through its watershed. The goal of this study is to evaluate the fate and impact of MP ingestion using experimental exposure of the common sole, *Solea solea*, a representative species of the Seine Estuary. Larvae of fish (from 7 days post hatch (dph) to 42 dph) were exposed during 2 hours to MP particles before (7 dph), during (21 and 22 dph) and after metamorphosis (41 and 42 dph). Three types of industrial MP were chosen with different properties (middle size floating (27-35 $\mu$ m, d=0.99), middle size sinking (27-32 $\mu$ m, d=1.20) and large size sinking (63- 75 $\mu$ m, d=1.20). In addition, at the last stage, fish were also exposed to natural plastic particles collected in the Seine River and ground into a fraction with a size less than 100  $\mu$ m (PVC, PE, PET, PS and PP tested separately). After exposure, fish which had ingested MP were counted (Figure 1) and isolated to monitor egestion. *Solea solea* ingested all types of plastics in variable (1 to more than 100) quantities and egested them on average less than 24 hours after exposure at all stages. A behavioural challenge, was performed on MP contaminated larvae. Behavioral responses of fish did not seem to be affected by MP exposures before metamorphosis compared to controls but distance moved decreased during metamorphosis for fish that had ingested MP particles. This could be explained by the fact that before metamorphosis fish can still use yolk energy reserve and are thus less affected by MP than later on when external feeding becomes mandatory.

Keywords: fish, solea solea, microplastics, behaviour

\*Speaker

# Preliminary results of plastic pollution analysis in the White, Barents and Kara Seas

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In recent years more and more studies concerning microplastic pollution in the Arctic are appearing: Lusher et al. (2015) showed first results of microplastic quantification in the area to the south of Svalbard, Ilka Peeken et al. (2018) proposed that Arctic sea ice can act as a sink for microplastics, Tekman et al (2017) showed a plastic contamination of the deep seafloor and van Sebille et al (2012) demonstrated that it is possible that a new garbage patch is forming in the Barents Sea and that plastic debris enter that area with Atlantic waters. However, there are still a lot of open questions and not enough in-situ data. Even fewer data are available in the Russian sector of the Arctic. In this study, we are showing preliminary results of the plastic contamination analysis performed during the "Arctic Floating University 2018" expedition in July 2018. A manta-trawl was used for surface sampling in addition to a bulk water sampling method. Quantification and classification of the microplastic candidates were performed under a magnifying binocular. Finally, the composition of the candidates was characterized using FTIR spectroscopy. The goal of the study was to quantify microplastic concentration in the White, Barents and Kara Seas. Besides analyzing microplastics in sea water, the study of the macro-litter on the shores of Novaya Zemlya was conducted using DeFishGear Methodology for Monitoring Marine Litter on Beaches. East and west shores were selected to compare the difference in marine litter accumulation between the Barents Sea and the Kara Sea.

Keywords: Arctic, sea water, in, situ data

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## How to get rid of ingested microplastic fibers? A demonstration by the shrimp *Palaemon varians*

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Coastal microplastic debris often contains high shares of fibers. These may originate from e.g.



brittle plastic ropes, fishing nets, or domestic effluents of textile washing. Various marine animals can ingest these fibers. However, the effects of microplastic fibers upon ingestion by marine biota are widely disregarded in ecotoxicological research. Therefore, we investigated whether microplastic fibers are eaten by the shrimp *Palaemon varians*, a common species in the coastal areas and estuaries of the North Sea. We investigated whether the shrimps differentiate between organic food and synthetic fibers and where the fibers remain upon ingestion. Animals were exposed for three hours to a suspension of 50 mg l<sup>-1</sup> of fluorescent acrylic fibers (ca. 150 – 300 µm) and different concentrations of food (0, 2.5, 5, and 10 mg per animal). After the feeding experiment, the digestive tracts were dissected, and the fibers were counted under a fluorescence binocular. *P. varians* readily ingested microfibers only when organic food was present simultaneously. Moreover, microplastic fibers ingested by *Palaemon varians* remained in their stomachs and did not enter the guts or midgut glands. One day after the feeding experiment, fibers appeared agglutinated in gel-like structures within the water but were absent in the feces of the animals. Ingestion of microplastic fibers by *P. varians* seems not to be an active process to satisfy hunger because the animals did not eat fibers when no food was present simultaneously. They rather ate the fibers by mistake together with their regular food. Further research is required to investigate whether the fibers were egested by regurgitation and, if so, whether this mechanism also appears in other marine organisms, representing an efficient way to eliminate fibrous microplastic debris from the digestive tract.

Keywords: ingestion, regurgitation, egestion, food

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## Microplastics in cosmetics in the italian market: an overview

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Microplastics - plastic material typically smaller than 5mm, derived from the fragmentation of larger objects or intentionally added to products – cause great concern because they are easily ingested by marine organisms. Microplastic fragments may not be well retained by filters and end up in the environment. As part of a project to raise awareness on marine waste, in 2017 the Clean Sea Life project (LIFE15 GIE / IT / 000999) carried out a survey to identify products containing microplastics currently on sale in Italy, mainly cosmetics and hand washing pastes for industrial use. The survey, conducted in 50 stores throughout Italy (perfumeries, pharmacies, supermarkets,

hardware stores) focused on polyethylene (PE) which, according to the European association of cosmetic manufacturers Cosmetics Europe, represents 93% of the microplastics contained in cosmetic products. The survey showed over 100 products containing polyethylene currently on sale in Italy, produced by 40 companies, some on sale in the "natural" products section. In this poster we present results of the filtration, drying and analysis (FT-IR, DSC, SEM, weight) of the solid residue of some of these products, carried out by CNR-ISMAR, which quantify the amount of polyethylene contained in each product. The project is also conducting an on-going evaluation to see if there is a change in the ingredient lists, and verified in some products the replacement of polyethylene with silica and perlite. Several EU Member States have enacted or proposed national bans on the intentional use of microplastics in certain consumer products, and the European Commission has expressed the intention, in the proposed Plastic Strategy, to take regulatory action at the EU level on intentionally-added microplastics to products of any kind. In Italy, a law that prohibits its use by 2020 was recently passed, thanks also to the results of the present study.

Keywords: microplastics cosmetics

\*Speaker

## In vitro effects of polyethylene microplastics on primary cultures and cell lines of fish

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Microplastic (MP) pollution in marine environments is in the spotlight due to the possible negative effects they can cause after their ingestion by marine fauna. Although ingested microplastics might harm fish health, current research has mainly focused on the toxicological effects of substances commonly found in marine water. Unfortunately, microplastics are able to incorporate these substances, with their subsequent accumulation and incorporation to the trophic food chain. In this work we aimed to investigate the effects of different polyethylene (PE) MPs on primary cultures of leucocytes and erythrocytes as well as to fish cell lines of the marine fish gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). Thus, head-kidney leucocytes (HKLs) from seabream or sea bass were incubated with 100 mg MPs/mL for 24 h. MPs higher than 20-25 µm. HKLs from sea bass showed significant mortality as well as up-regulation of the expression of genes related to apoptosis and cytochrome P450. No effects were produced in seabream HKLs nor in HKLs respiratory burst immune reaction. Regarding erythrocytes, no effect of MPs incubation was observed in the cell viability up to 1 week of incubation in vitro. Regarding

the seabream (SAF-1) and sea bass (DLB-1) cell lines the incubation with PE MPs failed to significantly affect their viability. In conclusion, it seems that PE MPs produce very little to no effects on cell cultures of fish cells in vitro.

Keywords: FISH, MICROPLASTICS, POLYETHYLENE, CELL LINES, LEUCOCYTES

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## Monitoring litter inputs from a river to the marine environment: Presence, diversity and seasonal trends in two beaches located close to its mouth.

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Marine litter, together with microplastics, is ubiquitous in the world's oceans and beaches, negatively affecting these, the economy, wildlife and human health. Despite the increasing interest and research related with marine litter, the progress that has been accomplished thus far, and the emerging studies regarding rivers as potential sources or vectors transporting it to the marine environment, seasonal trends of litter in beaches and the influence of rivers in these presence/absences are still to be unraveled. This work aims to fill some of the gaps related with this issue, studying the seasonal variation along a year and the influence of river incoming in the marine litter distribution in two beaches located in the Southwest of Spain (Atlantic Ocean). 55 categories of litter items were studied, in 5 different sampling sectors (3 in Valdelagrana beach and 2 in La Puntilla beach), 2-3 times per season, gathering 50 sets of data for 1 year. For this purpose, the existing sampling protocols were adapted and improved, in order to allow collecting data to be later on transferred to international databases. In parallel, the incoming to the sea due to the transport from the Guadalete River was evaluated weekly. The results show that in this specific case of study, the river is not the main source of marine litter for the beaches close to its mouth. Furthermore, it not even a remarkable source, due to most of this litter usually get stranded upstream in the meanders, being probably only transported to the ocean during extreme climatologic events (like river floods). Despite the categories of “cigarettes, butts & filters”, “plastic fragments” and “straws” were the most usually found in all sampling points, some specific temporal trends have been observed along the different seasons, according to the abundance and the importance in the total bulk of marine litter of the different categories studied.

Keywords: marine litter, river, beach, seasonal trends

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## Microplastic prevalence in the River Weser

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As global plastic production continues to increase steadily, so does the amount of synthetic material that enters aquatic environments. In recent years, more and more studies have focused on the occurrence of microplastics (MPs), i.e. synthetic organic polymers with a size < 5 mm, in the environment. These omnipresent and hardly degradable pollutants are easily accumulated in the environment and potentially ingested by a wide range of organisms which holds a high toxic potential. Whilst most studies focus on MP in marine environments, only very little data on MP pollution of river systems are available, particularly those focusing on estuarine regions as transition zones. However, riverine and estuarine systems may represent the main transport routes for MP, connecting terrestrial and marine systems.

This work aims at assessing MP pollution throughout the entire German River Weser, which connects big urbane and agricultural areas with the North Sea. Besides the major river compartments (sediment/water), the role of point sources, like waste water treatment plants, and diffuse sources (aeolian/drainage) are investigated. The riverine and atmospheric samples were collected in April and May 2018 while the outlet of two wastewater treatment plants will be sampled monthly over one year from July 2018 – July 2019. Samples will then be treated following a novel purification protocol in order to remove organic and inorganic residues. The isolated MP fraction will be analyzed with state-of-the-art methods (FTIR, RAMAN, PY-GC/MS) which provide polymer specific information about the particle count, size distribution and mass. Knowledge gained in this study will contribute to a better understanding of MP sources and pollution in rivers and will particularly aid in the understanding of transport mechanisms within interconnected aquatic systems, in addition to acting as a fundament for future conservation and monitoring measures.

Keywords: Microplastics, environmental samples, River Weser, Pyrolysis, GC/MS, FTIR spectroscopy

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# Fate and Transport of Particulate Plastics in a Pilot Scale Wastewater Treatment Plant (WWTP)

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Reports on the occurrence of particulate plastics (nano- and microplastic particles and fibers) in the environment emerge on a weekly basis, but quantitative data are still limited due to analytical difficulties and inconsistencies of the methods applied to detect particulate plastics in complex environmental matrices. Investigation of transport processes is therefore key to understand environmental fate scenarios and material flows from e.g. urban areas into the environment. While wastewater treatment plants (WWTPs) are considered hubs for particulate plastics between consumers and the environment, the fate of particulate plastics in the system is still highly uncertain and needs an accurate and truly quantitative assessment. In previous work, we synthesized nanoplastic particles and microplastic fibers, with a chemically entrapped metal tracer (Pd, In respectively), which enabled tracing plastic particles through well-established total elemental analyses. Here we used these materials to investigate the fate and transport of particulate plastic in a pilot WWTP representing the activated sludge process. Our findings show that > 95 % of the nanoplastic particles and microplastic fibers are associated with the sludge flocs and therefore removed from the wastewater stream by the activated sludge process. While the processes controlling retention of particles has been demonstrated before (e.g. engineered nanoparticles, etc.) and surveys of municipal treatment plants have shown similar trends in the magnitude of microplastics removed, we can now validate the retention of nanoplastics and microplastic fibers with a more complete mass balance study. With a better understanding of emissions from WWTP, one could suggest estimated annual load of particulate plastics released into the surface waters which could, by extension, be used as a starting point for fate modelling.

Keywords: wastewater treatment plants, nanoplastics, textile fibers

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# Toxicological effects of *Solea senegalensis* larvae after exposure to PCB contaminated microplastics

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Due to its ubiquity, plastic debris is nowadays recognised as an emergent contaminant of aquatic ecosystems. Microplastics (MP), i.e. plastic particles < 5 mm, are already described as the most abundant in marine waters. Besides primary MP, which can be released to the environment by accident or negligence, secondary MP are produced by fragmentation of post-consumer plastic products. Although, preliminary findings demonstrated that flatfish larvae ingest primary MP without significant pathological consequences (Fig.1), the potential effects of irregular MP or PCB contaminated MP, remains unclear. Senegalese sole larvae (8 dph) were exposed, via water, to four treatments of polystyrene MP, plus control: i) spherical MP (250 µm), ii) secondary MP (< 250 µm); iii) spherical MP contaminated with the mixture of PCB congeners (Aroclor 1254), and iv) secondary MP with Aroclor 1254. Two concentrations of every MP were employed: 1,000 and 10,000 MP·mL<sup>-1</sup>. The experiments had a total duration of 14 days to enable monitoring of full larval development (i.e. up to ocular migration). Larvae were fed daily with *Artemia* sp. metanauplii. At days 7 and 14, mortality and developmental anomalies (e.g. skeletal deformities, incorrect eye migration) were assessed and the remaining larvae from each treatment were collected and stored at -80°C for oxidative stress- and detoxification- related analyses (e.g. lipid peroxidation, and superoxide dismutase and glutathione S-transferase activities). The results were integrated to evaluate the potential effects and responses of fish larvae after MP exposure and to disclose the influence of MP as a potential vector of PCB toxicity.

Keywords: microplastics, polystyrene, flatfish larvae, Aroclor 1254, biochemical responses

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## Exposure of juvenile turbot (*Scophthalmus maximus*) to contaminated microplastic diet: Oxidative stress biomarkers, erythrocytic nuclear abnormalities and histopathological effects.

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In the last decades, the contamination and accumulation of microplastics (MPs) in aquatic ecosystems have been considered one of the major threats in terms of environmental wellness and human health. One of the main concerns of MPs relates to their predicted capacity of adsorbing hydrophobic organic compounds. This property can have a major impact in the persistence, horizontal transport and uptake of highly hazardous persistent organic pollutants (POPs). As a contribution to understand the risk of POP absorption to MPs, a controlled experiment was performed using juvenile turbot (*Scophthalmus maximus*) as biological model. The animals were exposed through feed to MP spiked with a mixture of polychlorinated biphenyl (PCB) congeners (Aroclor 1254), a well-known POP. Aryl hydrocarbon receptor-related metabolism, histopathology and genotoxicity were considered as major endpoints. Three different diets were prepared by incorporating commercial fish pellets with MP sizing between 200 and 250 µm: i) control, with uncontaminated MP; ii) MP contaminated with the PCB; iii) MP contaminated with the dehalogenated PCB. Moreover, a blank test was included to isolate potential effects of MP. The organisms were fed twice a day, and physico-chemical parameters of the water were monitored daily. At days 0, 14 and 36, twelve fish per treatment were sampled. Blood was used to survey erythrocyte nuclear abnormalities (ENAs). Depending on organ (gills, liver or brain), other analyses included biomarkers related to phases I-bioactivation and II-conjugation such ethoxyresorufin-O-deethylase activity, glutathione and glutathione-S-transferase activity. Oxidative stress biomarkers (superoxide dismutase activity, lipid peroxidation) and neuromuscular impairment (acetylcholine esterase activity) were also surveyed. Liver was also used for PCB bioaccumulation and histopathological analysis. The results were integrated in order to assess the influence of MPs as vectors of PCB and biofilm modified PCB toxicity in marine fish, a group that is known to be

particularly sensitive to these pollutants.

Keywords: Microplastics, polychlorinated biphenyl, biomarkers, genotoxicity, flatfish

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## Evaluation of a microplastic sediment separator in the context of MSFD beach microplastic monitoring

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Microplastics (< 5 mm) are widespread aquatic pollutants, observed in every marine compartments including sediments. The descriptor 10 of the Marine Strategy Framework Directive (MSFD) requires that the "properties and quantities of marine litter do not cause harm to the coastal and marine environment." To achieve or maintain Good Environmental Status (GES) in European Seas, European Member States (MS) have to develop strategies that should lead to programmes of measures. This involves the establishment of monitoring programmes, enabling the assessment of the status of marine waters on a regular basis. In order to obtain accurate assessment of microplastic abundance and its evolution in marine sediments, a monitoring protocol has to be set up. Part of this protocol, the routine extraction step is of first importance. The MicroPlastic Sediment Separator (MPSS) proposed by Imhof et al. (2012) is a commercialized tool reported by the manufacturer to reliably separate microplastics from sediment. In this work, we assess the MPSS potential for routine microplastics extraction from sediment. Performances, mainly efficiency and repeatability, were evaluated by varying test conditions (extraction times; sand grain sizes) with different microplastics in terms of sizes (from 0.1 to 5 mm), densities and shapes. The use of the MPSS for routine extraction of microplastics from beach sediment samples in the context of MSFD beach microplastic monitoring is discussed.

Keywords: Microplastic, sediment, extraction, MPSS, monitoring, MSFD

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# Adsorption rate study of persistent organic pollutants in function of physical and chemical degradation of marine microplastics

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Over marine microplastics they are adsorbed large amount of persistent organic pollutants (POPs), mainly polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OPs) and polychlorinated biphenyls (PCBs). These POPs have much more affinity for the plastics than seawater and therefore they tend to accumulate over microplastic surface. But this affinity and preconcentration is not the same for all kind of plastics. This study compare POPs adsorption rate over the most abundant plastics: Polyethylene Terephthalate (PET), High Density Polyethylene (HDPE), Polyvinyl Chloride (PVC), Low Density Polyethylene (LDPE), Polypropylene (PP) and Polystyrene (PS).

Moreover adsorption rate vary on function of physical and chemical degradation state of the plastics. POPs adsorption on microplastic is generated over the surface layer, mechanical friction of microplastic fragments (physical degradation) increase their surface and therefore their capacity to POPs preconcentration. But also, the chemical degradation of the plastic, measure by microplastic yellowness (Brandon 2016), varies the adsorption rates of these pollutants over microplastic, added to the fact that the most yellowish fragments are also the ones with more microfractures. In this study it has been evaluated on one side the adsorption rate of persistent organic pollutants (POPs) over different kind of plastic composition fragments, and on the other, the evaluation over the same kind of plastic (HDPE), with similar size and shape, but at different physical and chemical degradation conditions.

Keywords: degradation, diferent kind of microplastics, yellowness, POPs

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## Microplastics ingestion by deep-water catsharks *Galeus melastomus* and *Scyliorhinus canicula* (Chondrichthyes) in the Tyrrhenian sea (Western Mediterranean Sea)

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Nowadays plastic debris must be considered a ubiquitous element of marine ecosystems, distributed both in shallow and deep-water. Different diet studies suggest that shark and rays could be threatened by plastic ingestion. Blackmouth catshark, *Galeus melastomus* (Rafinesque, 1810) and lesser-spotted catshark, *Scyliorhinus canicula* (Linnaeus, 1758) are two abundant deep-water elasmobranchs, both opportunistic scavengers, whose feeding habits have been widely studied in overlaps and differences. The aim of our study is to verify any differences in frequency of plastic ingestion by the two species and to evaluate possible incidence according to the intra-specific variables. Samples result from a by-catch of professional fishing boat armed with trawl net for Giant red shrimps capture at depths between 400 m and 500 m. Morpho-anatomical data (total length, total weight, sex, maturity stage, weight of liver and gonads) were recorded for each individual. Stomach and intestinal contents were weighed before incubating separately in a 10% KOH digestive solution at 60° C overnight, to remove biogenic material and identify plastic debris using a stereomicroscope. Nature of suspected items was determined using Fourier Transformed Infra-Red spectrometry (FT-IR).

Preliminary results confirm that plastic ingestion occurs in both species and filaments-like are the most frequent items, without significant differences in the two catsharks. However, there is a greater variability in shape of plastics (sphere, fragment, film) ingested by *G. melastomus* than *S. canicula*. This probably reflects differences in feeding habits of the two species. The detection of plastics in the final part of the gastro-intestinal tract of both species seems to suggest that these animals can expel the plastics with the feces and no forms of gastric blockage have been observed. Studies are in progress to verify the magnitude of the observed differences between the two species and to better investigate the phenomenon.

\*Speaker

Keywords: shark, deep water, microplastic, Mediterranean Sea, ingestion

# The sorption potential of 9 pharmaceuticals on the surface of microplastics

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The interaction between environmental pollutants such as microscopic plastic particles (microplastics) and pharmaceuticals represents an unexamined concern related to intensively used chemicals, which pose a potential threat to the aquatic environment. Therefore, the current study investigates the sorption capacity of nine priority substances from the groups of anti-cancer drugs, beta-blockers, veterinary antibiotics and anthelmintics, on the surface of the most often identified microplastics in the aquatic environment. The sorption experiments were carried out in accordance with the OECD 106 procedure, using polyethylene (high and low density), polypropylene and polyvinyl chloride. The objectives include determining the sorption characteristics by analysing the aqueous phase samples at equilibrium adsorption using chromatographic techniques (HPLC-UV), as well as observing the influence of changes in pH and ionic strength on the process.

Keywords: pharmaceuticals, sorption, mikroplastic

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# Swimming behaviour as a new endpoint to assess microplastic effects on marine invertebrates

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Microplastics (MPs) are a global and emerging threat for the marine environment, due to their worldwide distribution and persistence. Literature data show that, once ingested, they do not seem to affect survival, but are able to induce sub-lethal effects in marine invertebrates. The aim of this study was to propose the swimming behaviour as sub-lethal endpoint for three zooplankton species, to assess MPs toxicity. The swimming speed alteration (SSA) of the rotifer *Brachionus plicatilis*, the brine shrimp *Artemia* sp. and the larvae (pluteus) of the sea urchin *Paracentrotus lividus* was investigated after exposure to polyethylene MPs. These invertebrates were exposed to a wide range of MP concentrations (from 0.01 to 10 mg/L) different in size (1-4 µm; 4-6 µm) for 24 and 48

hours. The sensitivity of the behavioural endpoint, measured with an automated recording system, was compared to mortality or immobility. The swimming behaviour was also evaluated after organism exposure to MPs spiked with benzophenone-3 (BP-3), a hydrophobic organic chemical used in cosmetics with direct input in coastal areas. MP build-up and significant differences in SSA were observed in all species exposed to virgin and spiked-MPs, while no acute toxicity was found in any invertebrates. Virgin MPs induced a higher effect on SSA than BP-3 spiked MPs in terms of LOEC, suggesting that BP-3 combined MPs are less toxic than MP itself. In general, these results show that short-term exposure to virgin and spiked MPs causes changes in behavioural response, affecting the swimming speed of marine rotifers, brine shrimps and sea urchin larvae. In conclusion, swimming behaviour can be a good tool to assess sub-lethal effects of MPs alone and in mixture with contaminants in marine invertebrates.

Keywords: swimming, invertebrates, sublethal toxicity, chemicals

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## Microplastic marine pollution: European policy needs

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Microplastics (MPs) are one of the environmental issues strongly debated by the public in relationship with marine pollution and human health. The awareness regarding this issue is also increasing at the policy makers level. Many international and intergovernmental bodies are debating about the global problem of plastics in the marine environment. However, the problem of microplastics has not yet been addressed globally in an appropriate manner by policy due to the complexity of the problem: dispersal, durability, diversified pollution sources, fragmented authorities, and uncoordinated policies. While it is easy to identify the recipient of the laws for microplastic production and product design, it is more difficult to identify the correct addressee for microplastics already released in the environment or coming from large items fragmentation. It is important to focus on which policy areas and what requirements are needed to promote actions for the management of microplastics. This poster provides an overview of the existing regulatory instruments, which address the topic of marine microplastics developed at European and

International levels. It discusses specific management options, measures, and best practices that are already implemented, underlining the existing gaps.

Keywords: microplastics, policy, marine pollution, legislation

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## Numerical modelling of plastic movement in coastal regions

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Recent investigations show that the largest concentrations of plastic litter in oceans occurs in the nearshore. Coastlines are a major sink of microplastics, yet it is unclear how these small particles are carried by breaking waves across the surfzone. The velocity fields of ocean models are used to predict the transport of microplastic around the world. Ocean models currently do not include coastal hydrodynamics and assume the plastic particles to behave as passive tracers. This makes these models incapable of representing processes such as non-linear shoaling and breaking waves as well as the sinking, inertial behaviour and the important process of beaching/resuspension of particles in the swash zone.

This work aims to combine the multi-layer non-hydrostatic free-surface model SWASH and Lagrangian tracking software PARCELS in order to predict the transport magnitude and direction of weakly inertial particles in the nearshore and in particular under the influence of breaking waves. We have successfully incorporated the capability to simulate inertial and buoyant particles into PARCELS and have validated the implementation by comparing to an analytical solution for the settling of weakly inertial negatively buoyant particles under linear waves (Eames; 2008). The comparison with the analytical solution is presented in Figure 1.

Current work focuses on the integration with PARCELS with the ultimate aim to simulate the beaching of plastics for various plastic types. This work will contribute to better understanding of

the behaviour of plastic particles in the coastal region where it directly affects humans, also it will contribute to a better understanding of plastic particle dynamics at the boundary of the ocean which will improve global scale plastic particle transport modeling.

Eames I 2008 Settling of particles beneath water waves J. Phys. Oceanogr. 38 2846-53

Keywords: Numerical Modeling, Microplastic, Coastline, Coastal, Waves

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## Microplastics in freshwater systems (southeast Poland) preliminary results

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Microplastic contamination is an increasing environmental problem. There are studies confirming the dominate role of rivers in plastics transport from terrestrial areas to the seas and oceans. In Poland, there is a lack of research concerning on the problems of microplastics in the freshwater environment. The research has been mainly related to the marine environment so far. Therefore, the purpose of the work is to check the presence of microplastic in freshwater system. This paper presents the preliminary results of the research on the occurrence of microplastics in surface water and bottom sediments as well as groundwater. Research was carried out in the southeast Poland, mainly in the city of Cracow. Two water samples from Vistula and Rudawa rivers and one sample of groundwater used as tap water were collected. The water samples were filtered through 40 µm glass fiber filter and then analyzed macroscopically. A stereomicroscope, SEM and FT-IR were used for further analysis. The presence of microplastics was not detected in surface water samples. In groundwater sample it has been found some particles, which suppose to be plastic debris. Additional analysis of that sample with SEM and FT-IR gave no certain results. All found debris are very similar to the microplastics.

Subsequent research applies to the bottom sediments. Three samples of sediments were collected from channels entering the Vistula River. Sediments were sifted through the sieve column. The sieve contents were poured with distilled water into porcelain dishes and allowed to dry in condition protected from possible air pollution. The collected, sorted sediment was subjected to a preliminary macroscopic analysis. Some fragment of a plastic film was detected. The reference sample was used as a standard to compare the results obtained from environmental samples. It was prepared using the cosmetic facial cleansers containing microgranules and shredded plastics.

Keywords: microplastic, freshwater systems, water, bottom sediment, Poland

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# Three-dimensional numerical simulation of microplastics dispersal from point sources in the Baltic Sea region

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Microplastics are found to be dispersed over a large geographical extend between continents, in oceans and on beaches (Barnes et al., 2009; Cozar et al., 2014). Depending on the processes present in an area, such as wind (Chubarenko & Stepanova, 2017), tides or water surface waves (Isobe et al., 2014), dispersal of plastic particles could extend over large areas, like the Baltic Sea (Chubarenko & Stepanova, 2017). Insight from these studies combined with three-dimensional numerical modelling are applied to the Himmerfjärden bay (near Stokholm, Sweden) and Oslo Fjord (Norway) to simulate the geographic dispersal of microplastics emitted from a point source (located at the outflow of a waste water treatment plant). A particular insight is the relative great distance of microplastics in the open sea dispersed by the wind (Chubarenko & Stepanova, 2017). In this regard, it was important to include wind in the numerical simulation. This research was carried out as part of the JPI Oceans WEATHER-MIC project (<http://www.jpi-oceans.eu/weather-mic/about>). The main physical processes that were taken into account in this research were as follows: water level fluctuations (i.e. tides, extracted from a larger numerical model of the Baltic Sea using the TPXO tidal database); tide generating and Coriolis force, and wind from the ECMWF database. In this early stage of numerical modelling, the microplastics dispersal was modelled with passive tracers in a Telemac 3D ([www.OpenTELEMAC.org](http://www.OpenTELEMAC.org)) simulation with a point source. A simulation representing 28 days shows a relatively small dispersal along the source point with the above mentioned processes implemented in the 3D models, which is explained by the sheltered source locations (Figures in Annex). Long-term simulations will be carried out and more processes, including weathering and biofouling, will be incorporated.

Keywords: three dimensional numerical model, microplastics, telemac 3D

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## Tracking plastic incorporation into biogeochemical cycles

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Recent studies show ubiquitous distribution and leaching of plastics in seawater, which increases bioavailability of plastic-derived DOM to marine organisms. In aquatic environments, many bacteria and fungi colonize plastic particles and utilize polymers as an energy source. We hypothesize that the utilization of plastics as a microbial carbon source can be traced in the isotopic composition of plastic-derived DOM (dissolved organic matter). As a derivative of oil products, this DOM will be depleted in  $\delta^{13}\text{C}$  and  $\Delta^{14}\text{C}$  signatures; this signal can be traced in microbial biomass. To explore the relationship between the microbial community and plastic-derived DOM, we use various isotopic approaches, i.e., isotopic tracer experiments in water, slurry and whole sediment cores, stable isotope probing (SIP) and nanoSIMS to track the incorporation of plastic derived DOM into the microbial loop. In this way, we aim to understand the fate of plastic particles in marine waters and sediment, their biogeochemical cycling and transport.

Keywords: microbial loop, carbon isotopes, plastic derived DOM, SIP, nanoSIMS

\*Speaker

## Citizen science: a useful tool for both microplastic research and environmental engagement

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Microplastics are currently one of the most widely distributed debris in marine ecosystems around the world. Over the past decade, microplastic detections have become a growing concern in the scientific community, that highlights the importance of a better understanding of their spatial-temporal distribution and the need for a society committed to face problems with a strong anthropogenic origin. The marine citizen science platform *Sea Watchers* monitors microplastic



abundance, distribution and composition in beaches involving educational community in scientific research in the framework of the 'Plastic 0' campaign. Here we present the preliminary results from several locations in the North Western Mediterranean coast. Citizen science may provide an important means to highlight environmental threats, to engage communities in environmental monitoring and to build on scientific and social connections. 'Plastic 0' involved more than 1500 students in beach sampling and microplastic analyses under training and mentorship of scientists. The results provided highlight the spatial-temporal variations in the beaches sampled, where densely populated points have reached maximum concentrations of over 40 items·m<sup>-2</sup>. 'Plastic 0' has proven to be a suitable project to involve a community in scientific research and foster private-sphere behaviors such as reduced plastic consumption, and public-sphere behaviors, such as stewardship activities.

## Microplastics in polychaetes from the Norwegian continental shelf. Methods for determination and preliminary results from analysis

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Our earlier investigations of sediments from the Norwegian Continental Shelf found a visual average concentration of 60 ± 80 mg microplastic/kg sediment (range from < LOD=0.001 g to 410 mg/kg), with a mean concentration confirmed by FTIR of 6 ± 10 mg/kg (range from < LOD=0.001g to 46 mg/kg). Microplastic concentration in polychaetes (*Galathowenia oculata*) from the same area are being investigated, herein methodology and preliminary results from these analysis are presented, and compared with analyzed sediments from the same stations.

The area of investigation is in the Northern North sea (close to the oil fields Kvitebjørn and Visund), and in the Barents Sea. The samples were taken from 66 to 80 m depth. Our method for investigation of the polychaetes is based on digestion, density separation, filtration and finally analysis with FT-IR microscopy.

The species and benthic ecosystem impacts of excessive levels of microplastics in polychaetes is currently unknown. Increased knowledge about the transfer of microplastic between the ocean, sediments and biota (in our case polychaetes) is vital to assessing consequences and addressing solutions for the global problem of microplastic pollution.

Keywords: microplastic, polychaetes, Norwegian continental shelf, benthic

\*Speaker

## Another top predator in the list: microplastics in Mediterranean Monk Seals

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Mediterranean Sea has been considered one of the areas with highest density of plastics. This is a particular Sea Region where one of the most critically marine mammal species, the Mediterranean monk seal, inhabit. The incidence of plastics, including microplastics, in this species has not been investigate yet. In this study we present the first results about incidence of microplastics in this predator through scat analysis. Scat samples from north Adriatic (Istria- Croatia, n=4) and south Tyrrhenian (Sicily-Italy, n=4) seas were collected and analyzed for both microplastics and dietary investigations. Microplastic separation and identification was carried out following previous methodology adapted for scat samples. In all samples, only demersal fish was identified, but only 30% of the samples contained microplastics (fibers). Although the study was carried out with limited sample size, the trophic transfer possibility is discussed. In addition, we underline the importance of this information for an endangered mammal such as the Mediterranean monk seal, and the use of scats for the first time for this species.

Keywords: Mediterranean Sea, Monk Seal, Microplastics, scats

\*Speaker

## Textual synthetic fibres from machine washing and tumble drying: emissions and their reduction

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Synthetic fibres released in cloth washing are assessed to be one of the major sources of primary microplastics into the environment. The objectives of this study is 1) to quantify the emissions of synthetic textile fibers discharged from sequential machine washings and tumble dryings and 2) investigate the collection efficiency of two commercially available tools that were designed to trap the textile fibres from washing waters. The synthetic fabrics under investigation included four

types of polyester textiles (two fleece fabrics, one softshell fabric and technical T-shirt), one technical T-shirt of polyamide and one jersey of polyacryl. All the fabrics were new/unused and they were separately treated in five sequential washing-drying cycles. The collection efficiency of Washing bag (GuppyFriend) and washing ball (Coraball) was determined. The three replicate samples from total washing water was filtrated and subsequently analysed gravimetrically and under an optical microscopy. The fibres released in the tumble drier were collected after each drying and their masses were determined. The number and mass of microfibrils released from test fabrics in the first wash varied in the range  $1.0 \times 10^5$  to  $6.3 \times 10^6$  kg<sup>-1</sup>, and 0.038 to 0.22 % w/w, respectively. The corresponding mass range in the first drying was 0.001 to 0.17% w/w. Fibre emissions showed a decreasing trend both in sequential washes and dryings. The ratio of machine washing to tumble drying varied between the fabrics: the ratio was nearly one or lower to polyester fleece fabrics whereas it was much larger to other tested textiles. The collection efficiency values for washing bag and Cora ball will be given and means to reduce the emissions will be discussed. For the further studies, sampling and analytical methods have to be developed in order to quantify these small microplastic fibres in the wastewater treatment plants and various environments.

Keywords: microplastic, polyester, polyamide, polyacryl, washing machine, tumble drier, emission reduction

\*Speaker

## Antioxidant and immune status of European sea bass after ingestion, combined or separately, of polyethylene microplastics and oxybenzone

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Microplastic (MP) pollution in marine environments is in the spotlight due to the possible negative effects they can cause after their ingestion by marine fauna. Although ingested microplastics might harm fish health, current research has mainly focused on the toxicological effects of substances commonly found in marine water. Unfortunately, microplastics are able to incorporate these substances, with their subsequent accumulation and incorporation to the trophic

food chain. In this work we aimed to investigate the effects, separately and in combination, of both microplastic (polyethylene, PE) and oxybenzone (2-hydroxy-4-methoxybenzophenone, BP3) on antioxidant status and immunological parameters of European sea bass (*Dicentrarchus labrax*). Fish were fed with diets consisting of control commercial diet alone or supplemented with PE MPs (100 mg/kg fish feed), BP3 (115 µg/kg fish feed) or PE+BP3 (BP3 adsorbed on PE MP particles). Liver superoxide and catalase activities were determined. Serum and head-kidney leucocytes (HKL) were obtained after 7 and 28 days from the beginning of the experiment, and innate immune parameters were studied (serum bactericidal activity against pathogenic bacteria as well as HKL phagocytic capacity and ability, respiratory burst and peroxidase activities). Results show some changes in both antioxidant and immune status of the sea bass immune system after ingestion of microplastics, in particular when they are combined with oxybenzone.

Keywords: Microplastics, polyethylene, oxybenzone, european sea bass

\*Speaker

## Developing baseline for future research on microplastic contamination in urban waters of Gujarat, through rapid evaluation of major water bodies in Ahmedabad

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Microplastics are now a threat to the natural systems and it has become a major pollutant. Plastic in natural environment, pollute the water resources, negatively affect the ecosystems and it acts a carrier of many pollutants like heavy metals, synthetic dyes and other toxic contaminants. For a nation like India which is the major consumer of plastic in the world, the awareness of microplastic is lacking and there is a clear evidence about the research gap in the field of assessments related to microplastics in India. The proposed study focuses on quantification and identification of major plastic types found in the water bodies in the urban area of Ahmedabad. The assessment is focused finding evidence of microplastic in Sabarmati river, 3 major lakes & ponds based upon different localities and the assessment was given for various plastic type like low-density polyethylene, high-density polyethylene, polypropylene, polystyrene, polyethylene terephthalate, polyvinyl chloride. The

assessment done with the help of phytoplankton net showed that there are evidences of plastic in the surface water bodies. Water samples were collected to assess microplastic through FTIR shows presences of microplastic as-well. The primary assessment of the various surface water bodies has shown significant amount of plastic and diversity in the types of plastic. The nature of the assessment was rapid the finding of the research provides a pathway to assess the actual impact and magnitude of contamination due to plastic in water bodies.

Keywords: Microplastic, Urban Environment, Lakes, Rivers, Synthetic Fibre, Rapid Assessment

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## Exploring public views on the issue of microplastics in relation to the recent government imposed plastic ban in western part of India: Perceived causes, perception of stakeholders and the degree of change in the understanding of the issue

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The government of Maharashtra, in India, announced a ban on manufacture, use, sale, distribution and storage of plastic materials such as one-time-use bags, spoons, plates, PET and PETE bottles and thermocol items. A similar ban followed suite in Gujarat, on 5th June 2018, on the World Environment Day. The civic authorities have imposed a fine of Rs 5,000, Rs 10,000 for the first-time and second-time offenders and a fine of Rs 25,000, along with a three-month imprisonment for third time offenders. This study was aimed at finding the perceptions of this ban on various stakeholders, their perceived cause and thereafter to check whether they can connect the microplastic crisis to this ban. Out of the 450 respondents that we interviewed or who participated in our study through a survey, which consisted of 160 NGOs, 148 local vendors who earlier used plastic, 33 vendors who earlier did not use plastic bags, 15 university researchers whose research is in the field of environment or allied subjects and 15 whose is not, 36 consumers and 43 school students. The questionnaire used was designed to be the same for all subjects and focused more on businesses as well as community and consumer perspectives. Only less than 59 respondents managed to correlate the plastic ban to the microplastics crisis, only 18 of these were able to do it

without prompted awareness from the questionnaire itself. The NGOs that worked with themes aligned with environment mostly showed that their understanding of the issue also considered the microplastics crisis, majority of which were influenced by the World Environment Day campaign against plastic. Others had varied perceptions. Finally we measured the degree of change of the understanding of the issue based on the responses, which resulted in a positive change of +2 out of a maximum of +5.

Keywords: plastic, plastic ban, microplastics, pollution, policy

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## Occurrence and identification of small plastic litter in Algerian beaches

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The lack of plastic recycling, the underdeveloped solid waste Management and people behaviour in Algeria has led to an important accumulation of plastic litter in the urban zones and the environment. Large plastic litter can be collected on the beach, unlike small plastic items, which remain in the sediment. Therefore, the aim of this work is to quantify and to identify the plastic particles ( $\geq 1$  mm < 60mm) existing on the beaches of the Algerian western coast. Furthermore, the aim was to find out the relationship between the plastic litter occurrence and human pressure in each beach. In this sense, anthropogenic impact was classified into high, medium, and low taking into account the distance between each beach and the nearest populated areas. Sediment sampling was performed within a 0.5 m x 0.5 m quadrant for each beach using transects set between the coastline and the dune system. Data analysis was based for each sample on the concentration of items (number/m<sup>2</sup>), and weight (g/m<sup>2</sup>), and size (mm). The mean weight ranged from 0.01 to 0.07 g/m<sup>2</sup>. A positive correlation between plastic items concentration and human pressure has been proved for three beaches: Sablettes (66 items/m<sup>2</sup>); Benabdelmalek Ramdane (34,29 items/m<sup>2</sup>), and; Petit Port (East, 66,4 items/m<sup>2</sup>). Microplastics (< 5 mm) composed of fragments and granules were dominant for all beaches except for Bahara (34%). In addition, a subsample of the plastics representing each beach was tested to characterize polymers and FTIR results indicated that 79.5% of the plastics were polyethylene and 15.4% were polyamide. The presence of plastic waste could be reduced on the beaches by setting more garbage containers, especially during the summer holidays,

and improving the waste management treatment. Recycling and environmental consciousness could be an efficient solution to minimize the plastic issue in Algeria.

Keywords: Plastic litter, Algeria, beaches, FTIR

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## Performance of bio-degradable plastic in the marine environment

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Replacing conventional plastic with biodegradable plastic is discussed as a possible mitigation strategy for marine plastic pollution. However, there have been hardly any systematic field tests under marine conditions. There is little information and a lot of bias. Some laboratory tests have been performed with natural marine matrices, few tests have been conducted under natural conditions in the field. Thus, there is not a lot of specific information about the performance of biodegradable plastic under marine conditions. Biodegradability is usually tested in a laboratory test in closed systems by following the CO<sub>2</sub> production and/or the O<sub>2</sub> consumption over time. Various standard laboratory tests exist for compost, soil and also marine conditions. Once proven in the laboratory, it is important to verify whether the material is also biodegrading under natural marine conditions. We have been developing in-situ tests for several marine scenarios, and tested about 20 different polymers and blends of plastic film with 12 - 100 µm thickness in natural coastal habitats in the Mediterranean Sea and Southeast Asia. All tested materials that have been proven biodegradable in lab tests showed disintegration in field tests. The half-life of materials range from some weeks to some years. The disintegration of all biodegradable plastic tested is much faster than estimated for conventional plastic. The disintegration depends on climate zone and habitat conditions, such as matrix (water, sand, mud), temperature and nutrients. Some habitats in the same region are more active in the biodegradation of a specific material. Different materials are degraded differently under different conditions, especially with regard to oxygen availability, i.e. apparently some materials degrade faster without, others faster with oxygen. Results of concluded and ongoing field experiments as a baseline for a catalogue of the specific half-life as a material property for the most common biodegradable polymers and blends are given.

Keywords: biodegradable plastic, disintegration, in situ tests, half life

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## Microplastics presence in Fuerteventura beaches (Cofete and Gran Tarajal)

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Marine debris impacts and microplastics presence on beaches has been widely reported. However, only one study had been performed by Baztan et al., 2014 in Fuerteventura Island. This new study, performed along 5 months allows a wider vision of microplastics impact on Fuerteventura beaches. For this study, two beaches of Fuerteventura were sampled, Cofete and Gran Tarajal. Beaches were chosen according to geographical orientation and human impact. Beach orientation allows to determinate impact of Canary Current on microplastics presence, whereas human use allows to determinate which if debris comes from the ocean or due to human activities. In addition, Gran Tarajal beach is located at the mouth of a stream bed, which flow debris from inland after strong rains.

Sampling was carried out between October 2016 and February 2017, according to the methodology developed by Baztan et al., whit some modifications. A 100 meters transect was chosen at the highest tide line, and triplicates samples were token along this transect. 3 adjoining quadrants were determinate for macro (> 5mm), meso (5mm – 1mm) and micro (< 1mm) plastics sampling. For meso plastics analyses, sand samples were dried at a stove, weight and sieve trough a sieve tower. For micro plastics, samples were dried at a stove and dry weight was recorded. Afterwards, based on Thompson methodology (Thompson et al., 2004), hipersaline water was added and mixed for two minutes, allowed to settling and upper layer was removed with a syringe and filtered through a 0.7 µm paper filter. Same process was repeated 3 times.

Results showed that Cofete beach, at the West side of Fuerteventura is more affected by marine debris contamination, due to the effect of the Canarian Current. These results agree with those obtained by previous studies both in Fuerteventura and other islands of Canarian Archipelago.

Keywords: Fuerteventura, Microplastics, Beach, Sand, Cofete, Gran Tarajal

\*Speaker



# Detecting microplastics pollution in world oceans using radar satellites

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Plastic pollution in world oceans is estimated to have reached 270.000 tones, or 5.25 trillion pieces. This estimation however is largely based on sea surface trawling which requires a considerable amount of resource to provide data for upscaling estimates. The plastic accumulates in the ocean gyres, creating so-called "garbage patches". The largest garbage patches are located in the North Pacific, the "Great Pacific Garbage patch" and in the North Atlantic, the "North Atlantic Garbage patch". This plastic debris will disintegrate and degrade into microplastic and colonized by microorganisms which can create unique surfactants and bio-film. We hypothesize, that these surfactants, will affect the fluid dynamic properties of waves (change in viscosity and surface tension) and make them detectable by radar satellite. In this study we used Sentinel-1A, COSMO-SkyMed and TerraSAR-X radar satellite images from different dates. If possible, detection based on the use of radar would yield data that current detection methods could not match in terms of spatial and temporal resolution. At present, radar is not used for this purpose, we therefore explore its potential by considering the impact of plastic on ocean fluid dynamics. We are also considered the contextual image analysis, ocean geophysical products of the sea surface temperature, surface wind, chlorophyll, wave heights and direction. In addition, we started lab experiments under controlled conditions to test the behaviour of microbes colonizing the two most common marine pollutants, polyethylene (PE) and polyethylene terephthalate (PET). The analysis of the satellite images had shown that a combination of surface wind speed and Langmuir cells- ocean circulation pattern create a distinctive appearance of surfactants, sea-slicks and microbial bio-films. The preliminary conclusion of our study is that radar satellite images may be able to detect plastic pollution in the open oceans and this method can be extended to other areas.

Keywords: Microplastics, Surfactants, radar satellite

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# Exposure of polystyrene microplastics on +0 juvenile *Sparus aurata*: An assessment on ingestion and fatty acid metabolism

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In the last years, the presence of plastics debris in the marine environment has been a constant over time, arousing the interest of the scientific community. Different mechanical and physicochemical processes such as biodegradation, thermooxidative degradation, thermal degradation and photodegradation, transform these debris into small particles, microplastics (MPs), which are very difficult to remove it. Swallowing of the non-digestible particles increases the risk of bioaccumulation in marine organisms and may spread along the trophic chain. In this study, the influence of exposure to MPs in the fatty acid metabolism and their ingestion on +0 juvenile *Sparus aurata* was assessed using two MPs concentrations. The presence of microplastics on fish gut was evaluated by fluorescence microscopic and fatty acid analysis was performed by GC-MS. Preliminary studies showed the presence of microplastics in the gastric intestinal tract of all treatments. Exposure to MPs seems to arise effects in the profile of fatty acids, namely on PUFAs. Based on these results, it is possible to assume that the exposure and ingestion of MPs by +0 juveniles affects the lipid metabolism of these organisms.

Keywords: Microplastic, Fatty acid, Ingestion, *Sparus aurata*

\*Speaker

# Isolation of Plastisphere phototrophs and a look at the early stage plastic colonisation in the Mediterranean Sea

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Microbial community composition on marine plastic particles has been investigated after long-term incubation periods (weeks or months), whereas little is known about the early stage colonisation process (first few hours). Understanding the patterns of early stage plastic colonisation is important, because the first colonisers may have the ability to influence the dynamics of the biofilm and the Plastisphere formation. For instance, species with potential to degrade plastics require access to the plastic surface for biodegradation to occur, and whether this is achieved or not will likely depend on the initial establishment of the microbial community. The role of substrate surface parameters in relation to environmental factors in the early stages of microbial attachment to microplastics is likewise unclear (Oberbeckmann et al. 2018). Here I will discuss results of recent fieldwork on the Mallorcan coastline of the Mediterranean Sea, aimed at characterising early stage plastic colonisation as well as isolating a marine phototroph that is able to colonise plastic surfaces. The phototroph we discovered will allow us to establish a minimal but environmentally relevant, self-sustaining community of plastic-colonising marine micro-organisms enabling the detailed quantitative study of early stage colonisation dynamics within controlled settings.

Keywords: Plastisphere, plastic colonisation, marine microbiology, phototroph, isolate

\*Speaker

## Characteristics of conventional and bio-based polymers during sewage sludge treatment

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Microplastic in limnic systems is in the focus of current research. Possible entry paths are waste water treatment plants, where most of the quantified polymer particles (smaller than 5 millimetres) are eliminated and sewage sludge acts as sink. Here, previous studies typically compare particle number and size of a waste water treatment plant's in-/outflow and sewage sludge.

Within laboratory experiments, digested sludge has been spiked with a specific number of starch blend and polypropylene particles of different size. The experiments were designed to evaluate changes of material properties for conventional and bio-based plastic. Here, a model water serves as abiotic control that correlates in its composition with the electrolyte composition of the supernatant of digested sludge.

After preparation and extraction (according to the developed procedure for micro plastic in anaerobic environments by our group), the polymers were characterised with optical, spec-

trosopic, gravimetric, and thermoanalytical methods. Results clearly show alterations of starch blend – after 28 days approximately 15 % reduction in mass for those with a diameter of 3 mm in size and 25 % reduction in mass for those of 6 mm diameter (fig. 1). In contrast, no variations (after 28 days less than 1 % reduction in mass) could be observed for polypropylene particles. The chosen particle sizes of 3 and 6 mm diameter correspond to those that may be introduced into the digester by food packages as co-substrate

This research work is a first step in order to analyse degradation and compare variations in material properties of different polymers in digested sewage sludge. All studies took place within the frame of project "Strategies for the reduction of urban plastic emissions into limnic systems (PLASTRAT)", funded by the Federal Ministry of Education and Research (BMBF) as part of priority programme "Plastik in der Umwelt – Quellen, Senken, Lösungsansätze".

Keywords: polymers, anaerobic environments, sewage sludge, analysis, degradation

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## Are drifting microplastics entry vectors of alien species in the Canary Islands?

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Exotic, invasive, alien, non-native and harmful species are being transported around the world by drifting plastic debris, though not a shipping vector, has also been shown to cause transoceanic transfer of marine life. The introduced organisms include viruses, bacteria, spores, algae, microalgae, cyanobacteria and others, which have a global, ecological, social and economic impacts. From the ecological point of view, invasive species has been identified, in general, as the second greatest threat to global biodiversity after habitat loss. In the last years, the identification of microalgae and cyanobacteria species with a distant biogeographical distribution in isolated areas without any anthropogenic disturbances of the Canary Islands as for instance, *Meloneis mimallis* (Milos Island, Mediterranean Sea), *Procentrum panamense* (Martinique, Contadora, French Polynesia and Panama) *Prorocentrum sipanadense* (Sipadan Island, Malaysia), *Prorocentrum elegans* (Belize), *Cabra levis* (Sea of Japan, Russia), *Gambierdiscus caribaeus* (Caribbean Sea, Florida and Belize) suggests that there are several vectors and pathways for aquatic invasive species in the archipelago (ballast waters, aquaculture, sediment transport, etc.) In this study, an account of

suspect alien species found on microplastics, that may have been inadvertently introduced into the Canary Archipelago is presented. This fact suggests that drifting microplastics could be an important vector for aquatic invasive species in the Canary islands.

Keywords: Alien species, microalgae, HABs, Canary Islands

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## Are microplastics vehicles of bacteria or virus pathogens for marine bivalves?

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Microplastics (< 5mm) exhibit intrinsic features such as density, hydrophobicity, or high surface/volume ratio, that are known to promote microbial colonization and biofilm formation in marine ecosystems. Some studies investigating microplastic associated bacterial communities in marine ecosystems revealed specific bacterial assemblages in comparison to the surrounding seawater, and among these the *Vibrio* genus appeared as commonly detected in the microplastic fraction. The *Vibrio* genus comprises numerous pathogenic species for human and marine organisms, including juvenile oysters (*Crassostrea gigas*) in which they have been associated, together with the presence of a herpes virus (OsHV-1  $\mu$ var), to successive mortality outbreaks in France raising questions about the role of microplastics on pathogen population transport and disease transmission. To address such question, we immersed plastic pellets of three different polymers (polyethylene,

polypropylene and polyvinyl chloride) in an oyster farming area (Brest, France) (Figure) over a summer period covering mass mortality events. Pellets were placed at different tidal levels (water column, sediment, intertidal subtidal) and collected each week over a 4-months period. Real-time PCR was performed in DNA extracted from seawater, oyster and microplastics samples to detect and quantify *Vibrio crassostreae*, a virulent population involved in the oyster disease, and the herpesvirus. The presence in both oyster and seawater samples of these pathogenic agents was revealed concomitant to significant oyster mortality that reached up to 61%. Detection of these pathogens on microplastics will be discussed at the conference: come along and see for yourself!

Keywords: bacteria, microplastics, coastal ecosystem, real, time PCR, vibrios

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## Nanofragmentation of polyethylene and polystyrene macro-plastics under simulated environmental weathering (thermooxidation and hydrodynamic turbulence)

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Fragmentation of macroplastics into microplastics in the marine environment is probably the process that has generated most drive for development of the microplastics research field. It is thus surprising that the level of scientific knowledge on the combined oxidative weathering (UV or thermal catalyzed) and mechanical fragmentation is relatively limited. Furthermore, it has been hypothesized that plastic fragmentation plausibly continue into the nanoplastic size domains, but environmentally realistic studies is lacking.

Here is shown result from a laboratory simulation of hydrodynamic conditions relevant for the shoreline environment, and fragmentation of thermooxidatively pretreated polyethylene film and polystyrene macroplastic cubes, were studied over the course of a 4-day stirring experiment. Subsamples was filtered, with analysis in light microscopy with automated image analysis particle size distribution determinations and the nanoplastic size fraction was measured using nanoparticle tracking analysis. The results illustrate that fragmentation of the macroplastic objects is observed already after two days but more distinct after 4 days, with higher abundance for the smaller size fractions, which imply more release of smaller sizes, or fragmentation is several steps. The nanoplastic fraction show very high abundance released or fragmented for both polymers and higher

for day 4 than day 2. The conclusions is that nanofragmentation is important and understudied process, and that standardized test protocols for both weathering and for mechanical treatments mimicking realistic environmental conditions is needed and then further testing of the most common macroplastic materials to assess the rates and fluxes of fragmenting particles to micro- and nanoplastic fractions should be conducted.

Keywords: Plastics, Fragmentation

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## Evaluation of systems for reduction of fibre emissions from washing machines

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Recent studies indicate that microfibrils represent a significant part of microplastics found in the environment. It is generally accepted that washing and drying of textiles and clothes is the main source of microfibre emissions into wastewater streams. Fibre emissions have been evaluated in several studies although the full effect of factors involved is not yet fully understood.

To reduce microfibre emissions from washing machine a filtration system was developed to be installed on washing machine and a protocol to evaluate its effectiveness was developed.

Results from our tests show that available solutions have very different efficiencies: from virtually no effect to reductions exceeding 90 %. The most efficient solutions generally involve "linear" approaches where all water/fibres must pass a filter, whereas "parallel" solutions where water is randomly filtered were less efficient. The long-term efficiency under repeated use still needs to be evaluated. It should be understood that tests performed in various studies follow different protocols and that results differ depending on the conditions used. Eventually a standardized testing protocol will need to be developed.

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Keywords: Microfibers, Microplastics, Solutions

# Plastics in freshwater dams with human impacts: microbial colonization and distribution

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Plastic particles introduced into aquatic systems will rapidly develop microbial biofilms on their surfaces. Studies in marine environments and rivers show that the surrounding milieu is of key importance for this process. Much less is known about biofilm formation on plastic particles in stratified lakes, such as reservoirs intensely used by humans. We assumed that the opposed gradients of light and oxygen trigger the development of biofilms differing in thickness and microbial composition. We also hypothesized that a principally biodegradable polymer would be colonized more intensely than conventional polyethylene (PE). To test this, 25 µm thick foil pieces made of Ecovior and PE were exposed in Hassel pre-dam (Harz Mountains, Germany) during summer stagnation. The three depths covered epi- meta- and hypolimnion. After three and five weeks samples were withdrawn to study biofilm thickness, microbial biomass, community composition and polymer mass and structure.

Microbial biomass on both polymers did not differ significantly among epilimnion samples, although biofilm thickness measured using Confocal Laser Scanning Microscopy was slightly higher on Ecovior. Microbial biomass estimates based on two methods (phospholipid phosphate extraction and crystal violet staining) differed because the methods target different biofilm components and have different flaws. Microbial colonization of both polymers increased during the exposition especially in the epi- and metalimnion and it was constantly low and dominated by bacteria in the hypolimnion. Here, Ecovior was colonized preferentially, and it showed higher mass loss than PE. This indicates that Ecovior might be both a substratum for attached growth and a carbon source. Using ATR-FTIR-Spectroscopy, a chemical change of PE exposed in the hypolimnion was also detected, indicating weathering of the polymer. The distribution and sinking behavior of plastic particles in dammed water bodies is one focus of the project MikroPlaTaS, which will also address taxonomic and mineral compositions of plastic-associated biofilms.

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Keywords: Plastic, freshwater, dams, biofilm, microbial colonization



# Microplastic load from different land-based sources and reflection in abundances in coastal waters and sediment in the eastern Baltic Sea

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Microplastics in the aquatic environment are of global concern. The studies in the Baltic Sea are increasing to determine the amount, size, character and impact of microplastic. There are not many studies on land-based loads of microplastic covering besides water treatment plants also other potential sources and pathways of microplastic pollution. During the last years, we have taken samples from different wastewater treatment plants (WWTP) situated along the Estonian coast, the sea surface close to these WWTP outfalls to the sea and sediments in the nearby areas. Besides, we have sampled several rivers covering the areas where the river is passing through the forests, agricultural regions and cities. Microplastic samples are also taken from cargo harbours, shipyards and public beaches. We will demonstrate the measured amounts, character (fraction, fibre) and polymer types of microplastic from different land-based sources and compare the information obtained when sampling the sea surface with Manta trawl and sediments with Niemistö corer or grab sampler.

Keywords: Microplastic, freshwater, marine, land, based sources

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# Plastic litters from the coastal inlets of the northeastern Sea of Marmara: Awareness and some misapplications

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This study is essentially an analysis of the plastic litter on the shores of a Turkish city (Kocaeli), on the basis of a process executed with reference to OSPAR. Furthermore, an Microplastic awareness survey is carried out to assess and raise awareness on this issue. The study found that the most common types of plastic litter observed on the shore to be disposable water bottles (PET),

plastic bags (PE), packaging wastes and plastic cups (PE, Styrofoam-PS) etc., in the respective order of frequency. The local government of the region regularly cleans the sea and the shore. The municipalities collect tons of plastic litter from the sea surface and from coastal inlets, using the Established Barrier System in creek. Furthermore, even though the municipality regularly collects the trash deposited in the crowded shore areas of the city, people visiting the area for daily excursions still litter around. The monitoring activities in the area revealed mostly "new" single-use plastic waste which is often larger than 5-10 cm in size, as well as various pieces of smaller "eroded" plastic waste which the cleaning workers cannot effectively collect and which are potential sources of MPs. The majority of the waste observed in the area were found to be disposable plastics, and their existence cannot be overcome even through two rounds of collection per week. In this context, it is evident that the production and consumption of disposable products should be reduced substantially, and awareness-raising activities should be accelerated. Obviously this is a problem beyond the capabilities of waste collection operations of the municipalities. The results of the survey also draw attention to the same important problem.

Keywords: OSPAR, awareness, shoreline, microplastics, survey

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## Object detection for microplastic using deep learning on microscopic images

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We still lack a standard method of analysis in terms of assessing microplastics pollution, which is recognized as a major problem affecting the whole environment including the waters, biota, air, and soil. The studies so far engaged in the classification of the microplastics with reference to their sizes, shapes, colors and polymer types. The most common forms of microplastics found in water and wastewaters are fiber, particle, and film. The literature has yet to be enriched with an artificial intelligence application developed for the classification of microplastics. In this study, microplastics were extracted from the wastewater after several processes such as oxidation of organic materials, density separation and filtration. Then microplastics on the filter were examined under a stereomicroscope and images were taken. The images of microplastics found in wastewaters were subjected to convolutional neural network (CNN), a deep learning algorithm for object detection purposes. Doing so utilized convolutional neural network for the classification of the microplastics

as either fibers, particles, or films, based on microscope images.

Keywords: deep learning, convolutional neural network, microplastics, object detection, wastewater, shape

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## Macro and Micro(plastics) in the Environment of Some French Rivers

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It is now known that the vast majority of microplastics found in the seas and oceans originate from lands. In such a process freshwater environment (rivers and riverbanks) play a major role. It is therefore necessary to imagine the scenario that a used plastic, becoming a waste after use, will be found in the environment if it has escaped to a waste treatment stream. Its stay in the environment can persist for a very long time and this waste will then be exposed to a set of environmental constraints (UV, rain, wind, mechanical erosion, ...) which will continue and amplify its degradation, leading to its fragmentation. The work we have undertaken consists of collect and analyze the composition of microplastics in the surface waters of different french rivers (Allier, Charente, Loire, Touvre, etc.). To do this, we rely on citizen science operations, in particular thanks to the contribution of the babylegs sampling net [3,4], which makes it possible to multiply samples and analyzes. <https://www.researchgate.net/project/RiverP>

Keywords: Microplastic, Fresh water, Rivers, Plastic pollution, Citizen science

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## Comparison of microplastic content in digestive tracts of two marine vertebrate species in the Mediterranean: loggerhead turtles (*Caretta caretta*) and striped dolphins (*Stenella coeruleoalba*)

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Microplastics are becoming well documented in different environmental compartments. However, little is known about their presence in wild and non-commercial protected species yet. In this on-going study, we compare microplastic contents in digestive tracts of loggerhead turtles (*Caretta caretta*) and striped dolphins (*Stenella coeruleoalba*) stranded in Valencia region, Spanish Mediterranean. In total, we analysed 12 loggerheads recorded in 2017-2018, and 47 striped dolphins recorded between 1989 and 2017. Loggerheads had a mean $\pm$ SD= 8.25 $\pm$ 8.62 microplastic items per individual, while striped dolphins had 14.9 $\pm$ 22.3 microplastics per individual. Although microplastics seem to be more abundant in striped dolphins, this species showed higher variability. Concerning microplastic shape, dolphins had mostly fibres (73.3 %), with fragments representing 26.4 % of all items, while turtles similar amounts of both categories (fragments: 55.5%, and fibres 44.4%). No primary microplastics or pellets were found in loggerhead turtles, but we did observe two dolphins with white pellets, both located in close locations although stranded in different years. Regarding microplastic colours, both dolphins and turtles had black as predominant in fibres (68.4 and 60 %, respectively). Regarding colour of fragments, in dolphins, translucent was the most predominant colour (46.8%), followed by white (15.8%) and black (15.2%). In turtles, most of fragments were blue (30.9%), followed by white (27.3%) and translucent (20%). In conclusion, both species intake microplastics in the Mediterranean Sea and there are no big differences concerning microplastic shape or colour. Further research should focus on how they intake it, and whether its intake could affect their fitness. It is essential to continue monitoring in order to have better knowledge about the role of plastics in marine ecosystems as well as to be able to identify the most problematic plastics and act accordingly.

Keywords: striped dolphins, loggerhead turtles, microplastics, strandings, western Mediterranean

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## Marine bacteria colonize and enrich on plastic pellets

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Since the introduction of mass production in the beginning of the last century, plastic production has been growing continuously resulting in an estimated 8 million metric tons of plastic

waste annually entering the oceans. We are only in the very beginning of understanding the impact of plastic on the marine environment, including its transport, degradation and potential risk. Microorganisms could influence the fate of plastic debris by colonization or degradation. As a basis to investigate bacteria-plastic interactions, the purpose of this study was to develop a simple experimental setup to estimate bacterial colonization on plastic pellets and utilize it to identify plastic colonizers by enrichment. Using a bacterial isolate, we found that neither addition of nutrients nor incubation longer than 24 hours did have an effect on pellet colonization. Testing the setup with six bacterial strains and three common thermoplastics (polystyrene, polypropylene, polyethylene) demonstrated that only in the case of one strain, the plastic type influenced the level of colonization. Incubating natural seawater with plastic pellets for two weeks demonstrated that the communities on plastic were distinct from the initial microbial diversity present in seawater suggesting an enrichment of certain bacteria on the pellets. However, diversity did not differ depending on plastic type. Currently, the samples are investigated for the presence of genes involved in plastic degradation using degenerate primers. In the future, the setup will allow estimation of the impact of bacterial colonization on plastic transport in the aquatic environment as well as isolation and identification of possible plastic-degrading microorganisms.

Keywords: Plastic, Bacteria, Bacterial colonization, Marine ecosystems

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## CRISS project: an opportunity to integrate marine litter and micro plastics problematic within interdisciplinary projects, while working Digital Competence in the school.

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Competence Based Education (CBE) has a long history, but in the last decade, its presence in the European educational systems has become a reality. Despite this presence and the efforts, how to work in the school and performs the assessment following the CBE is still a challenge.

We introduce here some elements of the H2020 CRISS project. CRISS is a user-driven, flexible, scalable and cost-effective cloud-based digital learning ecosystem that allows the guided

acquisition, evaluation and certification of digital competence in primary and secondary education, and it is easily scalable to other educational levels. The transversality of the competences allows the integrations of other Key Competences within the same ecosystem, as “Mathematical competence and basic competences in science and technology” or “Social and civic competence”. CRISS provides a Digital Competence framework addressed to primary and secondary schools. It consists of five areas (digital citizenship, digital communication and collaboration, search and manage digital information, digital content creation, digital problem solving) that group 12 sub-competences. Digital Competence (DC) is developed and evaluated in context by means of Competence Assessment Scenarios (CAS) integrated into the school curriculum. The CAS pedagogical approach is based on the pedagogy of integration. A CAS adopts advanced instructional approaches where the learner or learners are at the center to solve problems or develop projects in realistic contexts and meaningful situations. CRISS provides a set of CAS, covering the whole DC, and gives the possibility to adapt them or to create new scenarios. This flexibility allows to embed every topic in an interdisciplinary way. As an example, we present one of the CAS: "Through the Screen, and What Alice Found There", whose objective is invite the students to participate in an active mood through digital technologies in social improvement and environmental sustainability initiatives related to MP's.

Keywords: Competence Based Education, Digital learning, Marine litter, Interdisciplinarity

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## A protocol to successfully extract DNA from different polymers to investigate microbial diversity and the capacity of polymers to spread harmful marine micro-organisms

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Plastic debris found in the marine environment might serve as vectors for dispersal of harmful micro-organisms and invasive species. Despite recent research on plastic-associated micro-organisms in seawater, very few studies investigated simultaneously the presence of harmful marine micro-organisms and microbial communities on polymers with different surface properties and composition. The detection of target micro-organisms and the molecular characterization of communities are highly impacted by the composition of polymers and by technical and analysis method. In this respect, DNA extraction is a challenging step since it has a crucial effect on the determination of community composition. In the present study, six DNA extraction methods were selected to evaluate microbial colonization of three types of polymer immersed in coastal seawater of the Bay of Brest (France, August 2017). The three polymers (Low density polyethylene, polyamide and polypropylene membranes (30 cm<sup>2</sup>)) were collected after 15 days of immersion and DNA was extracted. DNA yield, integrity and purity varied among methods.

If all the tested methods led to successfully amplify the 16S and 18S rDNA, control of DNA amplification inhibition with real time PCR for *V. parahaemolyticus* including an exogenous internal positive control showed differences between protocols. Strong inhibition with the (Phenol/Chloroform/isoamyl alcohol (PCI) protocol was observed when using sucrose in the preliminary lysis step. Target PCR indicated the presence of total *Vibrio* spp., *Vibrio alginolyticus*, *V. parahaemolyticus*, *Alexandrium minutum* and *Marteilia refringens* on the three types of polymer. Other target taxa, *Ostreopsis* spp., *Bonamia* spp. and the herpesvirus, OsHV-1, were not amplified. The microbial community composition was then examined using a metabarcoding approach spanning the V3-V4 regions of the 16SrRNA gene. The results will be discussed enlighten the pros and cons of the selected protocols.

Keywords: DNA extraction methods, plastic debris, microbial communities

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## Comparative weathering of the nine polymers that constitute the 'Baseman's Kit'

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There has grown a general awareness in the scientific community that identifying a piece of a polymer in an environmental sample requires not only a background database with pristine polymers to compare with but an evaluation of its potential degradation. Despite a ‘quantitative’ assessment of the degree of evolution of a polymer in the environment would be ideal, this is so far almost impracticable due to the very many difficulties in deriving generalized conditions. Therefore, we need at the very least to include in the databases general patterns that apprehend how the polymers evolve during their natural weathering. For this, a weathering system emulating the solar irradiation at the Earth surface was deployed; marine and dry-shoreline conditions were simulated and the nine polymers constituting the BASEMAN’s kit of microplastics were weathered for three months.

In this work a general comparison is given for the weathering of the nine polymers that constitute the so-called BASEMAN’s kit of polymers for studying of the presence of microplastics in the oceanic environment. The most relevant infrared (medium region) spectral bands giving rise to the major differences between pristine (as received) and weathered polymers are described.

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Keywords: Microplastic, Weathering, Infrared Spectroscopy

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## A Comparison between chemical and enzymatic digestion for the analysis of microplastics in plankton

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Plastic is one of the most frequent materials worldwide and constitutes one of the most common and persistent pollutants in the oceans. In fact, plastics amount up to 60-80% of total marine litter. A particular fraction of plastic debris is constituted by the microplastics (particles 5 mm). The primary risk associated with microplastics is their bioavailability to marine organisms, including phyto- and zooplankton, which can ingest microplastics. When this occur, microplastics might



obstruct feeding appendages, aggregate and block the alimentary canal, therefore limiting the natural food intake of those organisms or, even, be translocated into the circulatory system. In addition, microplastics can introduce pollutants into the organisms, either additives from their production process and/or hydrophobic contaminants present in the marine environment. Very scarce reports have been found in literature reporting on a complete digestion of plankton. There are two main types of digestion: chemical and enzymatic. In the former, one or more chemicals (KOH, H<sub>2</sub>O<sub>2</sub>) are used to dissolve (digest) the biomass, being one of its major drawbacks that it could destruct the plastic particles and their surfaces and create interferences that difficult their infrared microspectroscopy measurement. Thus, enzymatic digestion methods are proposed to minimize damage to plastics.

In this work, an enzymatic and a chemical digestion have been developed and optimized to digest biological material and, further, identify and characterize the microplastics by optical microscopy and  $\mu$ FTIR.

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Keywords: microplastic, enzymatic digestion, chemical digestion, plankton

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## Direct determination of antimony in microplastics by ultrasonic slurry sampling and electrothermal atomization atomic absorption spectrometry

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Additives are included in plastics for various purposes, as fillers to reinforce plastic resin, thermal and UV/light stabilizers, flame retardants, pigments, antimicrobials, etc. Also, they can be incorporated in plastics as catalyst residues from the polymerization processes. Several metals are part of their formulation, namely lead, zinc, iron, titanium, antimony, aluminum, calcium, barium, copper and nickel. Antimony compounds, particularly Sb<sub>2</sub>O<sub>3</sub>, are typically used as polycondensation catalysts in PET polymerization, as synergist flame retardant compounds in

halogen containing plastics and also as pigments, due to their resistance to heat, light and chemical. Thus, Sb is present on a variety of plastics and therefore it can be discharged into the oceanic environment from plastic residues like the microplastics. This metal is considered by the USEPA and EU as a priority pollutant and, from the environmental point view due to its natural low levels in the earth's crust, it can be considered as a potential marker of environmental metallic pollution.

In this work the direct determination of Sb in microplastics was accomplished by automated ultrasonic slurry sampling (USS) and electrothermal atomic absorption spectrometry (ETAAS). The experimental conditions for sample homogenization, the instrumental parameters and the furnace temperature program were carefully optimized. The use of a surfactant (Triton) was revealed necessary to promote the suspension of the microparticles. Microplastics samples of PET, PVC, LDPE, PC and PS were analysed.

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Keywords: Microplastic, plastic additives, antimony, automated ultrasonic slurry sampling, ETAAS

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## The use of Polysorbate 20 as an "optimizer" component in the alkaline digestion of fatty fish digestive tract for microplastic extraction

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The ubiquitous presence and accumulation of microplastics in the ocean and inside different marine organisms have become a global concern. Alkaline digestion techniques using potassium hydroxide (KOH) are currently accepted and applied in monitoring studies to separate microplastics from biological samples. The KOH allows an efficient digestion of organic matter without degrading plastic polymers in a time- and cost-effective way. However, for some fishes which contain large amounts of fat, alkaline approaches were found to be troublesome, due to the formation of fat layers which difficult the digestion and solubilization of biological material. To overcome these limitations, this study aims to evaluate the influence of Polysorbate 20 and stirring in the known alkaline protocol. The potential effect of stirring and Polysorbate 20 on eight common

polymer types were searched by Fourier-Transform Infrared Spectrometry (FTIR). Digestion efficiency was assessed using ray's liver which is known to be a tissue with a high fat content. The results indicate that Polysorbate 20 and stirring can be used with no interference or minimal interference in the FTIR spectrum of the majority of microplastics. Moreover, for increasing lipids emulsification, it provides a less viscous solution that facilitates the filtration step and increase the recovery rates of microplastics. In order to assess the efficiency of the optimised protocol for environmental samples, 40 digestive tracts of ray (*Raja* spp.) from the Portuguese coast. Samples were processed using a solution of 10% KOH with 10% Polysorbate 20 for 24h at 60°C, filtered with a cellulose nitrate 12 µm filter and analysed by stereo microscope and FTIR.

Keywords: Polysorbate 20, ray, microplastic, methodology

The use of Polysorbate 20 as an “optimizer” component in the alkaline digestion of fatty fish digestive tract for microplastic extraction. Clara Lopes et al., > 222617

## The use of Polysorbate 20 as an “optimizer” component in the alkaline digestion of fatty fish digestive tract for microplastic extraction

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The ubiquitous presence and accumulation of microplastics in the ocean and inside different marine organisms have become a global concern. Alkaline digestion techniques using potassium hydroxide (KOH) are currently accepted and applied in monitoring studies to separate microplastics from biological samples. The KOH allows an efficiently digestion of organic matter without degrading plastic polymers in a time- and cost-effective way. However, for some fishes which contain large amounts of fat, alkaline approaches were found to be troublesome, due to the formation of fat layers which difficult the digestion and solubilization of biological material. To overcome these limitations, this study aims to evaluate the influence of Polysorbate 20 and stirring in the known alkaline protocol. The potential effect of stirring and Polysorbate 20 on eight common polymer types were searched by Fourier-Transform Infrared Spectrometry (FTIR). Digestion efficiency was assessed using ray's liver which is known to be a tissue with a high fat content. The results indicate that Polysorbate 20 and stirring can be used with no interference or minimal interference in the FTIR spectrum of the majority of microplastics. Moreover, for increasing lipids emulsification, it provides a less viscous solution that facilitates the filtration step and increase the

recovery rates of microplastics. In order to assess the efficiency of the optimised protocol for environmental samples, 40 digestive tracts of ray (*Raja* spp.) from the Portuguese coast. Samples were processed using a solution of 10% KOH with 10% Polysorbate 20 for 24h at 60°C, filtered with a cellulose nitrate 12 µm filter and analysed by stereo microscope and FTIR.

Keywords: Polysorbate 20, ray, microplastic, methodology

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## Chronic effects of polystyrene microplastics on two crayfish species

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As the world continues to focus in on microplastic pollution of the environment, it becomes increasingly necessary to better understand the environmental risks surrounding these particles by adding to existing knowledge. The effects on biota are one of the key components of this work. The use of consistent testing protocols has so far been limited, potentially reducing the robustness of any risk assessments derived from different studies. This study closely follows other experimental designs for testing microplastics with other benthic macroinvertebrates with the aim of contributing to the taxonomic diversity of tested species and ultimately being a basis for an environmental risk assessment. Due to their additional role as a human food source, two crayfish species were used (*Procambarus acutus* and *Pacifastacus leniusculus*) and tested as juveniles, the standard set up previously used was altered to accommodate their idiosyncrasies. Each bioassay held an individual organism for 28 days with microplastic spiked sediment. A wide range of polystyrene microplastic concentrations were used in the sediment: varying from no microplastic, starting at environmentally relevant concentrations (0%, 0.1%, 1%) through to high levels (5%, 10%, 20%, 40%). For each of the seven concentrations eleven replicates were used. No effects of microplastic spiked sediment were seen on the survival or weight for either *Procambarus acutus* or *Pacifastacus leniusculus*. The high concentrations used in the sediment compared with concentrations found in the field gives a good indication that microplastic pollution has limited effect on the growth or mortality in crayfish species.

Keywords: Polystyrene, microplastic, crayfish, effects, chronic. \*Speaker

# Marine plastic pollution as a planetary boundary threat – The drifting piece in the sustainability puzzle

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The exponential increase in the use of plastic in modern society and the inadequate management of the resulting waste have led to its accumulation in the marine environment. There is increasing evidence of numerous mechanisms by which marine plastic pollution is causing effects across successive levels of biological organization. This will unavoidably impact ecological communities and ecosystem functions. A remaining question to be answered is if the concentration of plastic in the ocean, today or in the future, will reach levels above a critical threshold leading to global effects in vital Earth-system processes, thus granting the consideration of marine plastic pollution as a key component of the planetary boundary threat associated with chemical pollutants. Possible answers to this question are explored by reviewing and evaluating existing knowledge of the effects of plastic pollution in marine ecosystems and the ‘core planetary boundaries’, biosphere integrity and climate change. The irreversibility and global ubiquity of marine plastic pollution mean that two essential conditions for a planetary boundary threat are already met. The Earth system consequences of plastic pollution are still uncertain, but pathways and mechanisms for thresholds and global systemic change are identified. Irrespective of the recognition of plastic as a novel entity in the planetary boundaries framework, it is certain that marine plastic pollution is closely intertwined with global processes to a point that deserves careful management and prevention.

# Bisphenol A and its effect on photoautotrophic and heterotrophic organisms

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Bisphenol A (BPA) is an environmental contaminant that is present not only in soil, but also in aquatic ecosystems. Hence, it is important to study the potential genotoxic activity on organisms that naturally occupy these habitats. In our research, we studied the toxic effect and potential genotoxicity of BPA on algal and yeast cells. As model organisms *Chlamydomonas reinhardtii*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* were used. We found out that increased

concentrations of BPA affected the growth rate of cells and inhibit cell division.

Keywords: bisphenol A, growth rate, toxicity/genotoxicity, yeast, algae.

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## Bisphenol A as an environmental pollutant with genotoxic effect on human lymphocytes

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Bisphenol A (BPA) is widely used in industry to manufacture polycarbonate plastic and epoxy resins and serves for production of plastic bottles, food cans, containers, thermosensitive paper, dental fillings and other medical aids. It was found that BPA belongs to endocrine disrupting chemicals (EDCs) or exogenous substances that can alter the function of the human endocrine system. For its large production and poor recycling, it has become a new environmental pollutant. In our study, the potential genotoxic activity of BPA on human lymphocytes was evaluated by using the alkaline comet assay. Moreover, to detect oxidative DNA damage in human lymphocytes a modified comet assay with bacterial DNA repair enzyme FPG was used. Our results show that increasing concentrations of BPA induce DNA double-strand breaks and oxidised purines in human lymphocytes. Thus, the proposed mechanism of BPA action in human lymphocytes could be mediated by induction of ROS.

Keywords: bisphenol A, comet assay, oxidative DNA damage, ROS

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# Impact of microplastics alone or with adsorbed oil compounds from the water accommodated fraction of a North Sea crude oil on marine mussels *Mytilus galloprovincialis*

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Due to their hydrophobicity and relatively large surface area, microplastics (MPs) can adsorb persistent organic pollutants such as oil hydrocarbons present in the ocean and may facilitate their transfer to organisms (the so-called "Trojan horse effect"). This study examined the effects of dietary exposure to polystyrene MPs of 4.5 µm alone and with adsorbed oil compounds from the water accommodated fraction (WAF) of a naphthenic North Sea crude oil at two different dilutions (25% and 100%) on mussels *Mytilus galloprovincialis*. Pristine or contaminated MPs were provided daily mixed with algae, at an environmentally relevant concentration of 1000 particles/mL. Additionally, an extra group of mussels was exposed to a 25% dilution of WAF for comparison purposes. After 7 and 21 days of exposure, 16 EPA PAHs were determined in mussel whole tissues; catalase activity, neutral red (NR) uptake and DNA damage by comet assay were determined in hemocytes and activity of antioxidant enzymes and histopathology were assessed in the digestive gland. Whole organism responses (condition index, oxygen consumption, clearance rate, absorption efficiency (AE) and scope for growth (SFG)) were measured after 21 days of exposure. Significant accumulation of PAHs occurred in mussels exposed to WAF but not in those exposed to MPs with adsorbed WAF compounds. Accordingly, NR uptake decreased significantly in hemocytes of mussels exposed to WAF with respect to controls (Figure 1). At whole organism level, AE and SFG decreased in the same group. Single strand breaks in DNA increased in all treatments compared to controls. Further studies are in progress to unveil the potential role of MPs as carriers of oil compounds.

Keywords: Polystyrene microplastics, Water accommodated fraction of oil, Dietary exposure, Trojan horse effect, Cell and tissue biomarkers, Scope for Growth, Mussels.

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Microplastics in the Portuguese coastal waters. Clara Lopes et al., > 222616

## Microplastics in the Portuguese coastal waters

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Nowadays, plastic has achieved a key status in the society, with applications in different sectors such as in the commerce, industry, and medicine. Plastic debris, which may be accidentally lost or intentionally rejected, tend to accumulate in coastal areas, being a threat to marine organisms. Plastic marine debris drifting in the water column gradually breaks into small fragments, called microplastics (< 5mm), being bioavailable to be ingested by several organisms. Transects, perpendicular to the coast, were made along the Portuguese coast during May 2018 with the NI Noruega. Samples were collected at the end of the day using plankton nets with a mesh size of 300 µm. The nets were towed during 45 minutes near the surface with a constant ship velocity. Samples were frozen until further analyses. In the laboratory, samples were sieved with a 1 mm mesh. The part of the sample that pass through the sieve was then filtered with a 20 µm polycarbonate filter and observed with a stereo microscope. The part of the sample that was larger than 1 mm was processed and observed under a stereo microscope. The results indicate fibers as the predominant category of microplastic followed by fragments. Higher microplastic concentrations were observed between Peniche and Sines, probably associated with the presence of the Tagus estuary, the largest in Europe. This area also has high population densities and industrial activities that promote the enter of this anthropogenic pollutant in marine environment.

Keywords: microplastics/water column/coastal area/Portugal

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## Individual and mixture exposures of polyethylene, polypropylene and polystyrene micro- and nanoplastics to freshwater microbial communities

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Micro- and nanoplastics, defined as < 5 mm and < 1 µm respectively, are recognised as global



environmental contaminants. Due to their prevalence in aquatic environments, inhabiting organisms frequently encounter them, resulting in detrimental effects to the organism's health and survival. Laboratory studies often focus on single polymer type exposure when assessing potential impacts of plastics. However, micro- and nanoplastics exist as a "cocktail of compositions" within the environment. Analysis of the effects of a single polymer type may miss any synergistic effects that arise from exposure to mixtures of different micro- and nanoplastic compositions; thus results from single composition studies may not be representative of the more complex effects in the environment. In this study, freshwater microbial communities, taken from surface waters of the Worcester and Birmingham canal, were exposed to polyethylene, polypropylene and polystyrene particles of varying sizes (200 nm, 3  $\mu$ m and 30  $\mu$ m) and concentrations (0.01, 0.05 and 0.1 mg/L) either singly, as pairwise mixtures or as a mixture of all three compositions. Using Biolog<sup>®</sup> multiwell plate technology, active respiration of the microbial communities was measured to ascertain whether the microorganisms were capable of surviving the exposure conditions and utilising the polymer(s) as the sole carbon source over 72 hours. Surface characteristics of the 3 polymers were also examined via Scanning Electron Microscopy before and after exposures to observe any evidence of microbial colonisation or biodegradation. By incorporating micro- and nanoplastic mixture exposures into laboratory-based studies, any observed impacts to microbial respiration or the plastic surfaces could more accurately represent in-situ environmental processes. Furthermore, freshwater research remains an understudied area in comparison with the marine environment. Therefore, effort needs to be directed into bridging the gap between the two environments and also between what is happening in the environment and what we are observing in laboratory experiments.

Keywords: Freshwater, Microbial communities, Toxicity, Mixtures

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## FATE AND IMPACTS OF MICROPLASTICS: KNOWLEDGE, ACTIONS AND SOLUTIONS

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