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Local variation within marinas: effects of pollutants and implications for invasive species

Joseph M. Kenworthy¹, Guillaume Rolland¹, Sarah Samadi² and Christophe Lejeusne^{1,*}

1. Sorbonne Université – Université Pierre & Marie Curie (Paris 06), CNRS, UMR 7144 AD2M, Station Biologique de Roscoff, Place Georges Teissier CS90074, 29688 Roscoff, France.
2. Muséum national d'Histoire naturelle, Sorbonne Universités, ISYEB (UMR 7205 CNRS, MNHN, UPMC, EPHE), CP26, 57, rue Cuvier, F-75005 Paris, France

*corresponding author

JMK: joseph.kenworthy@sb-roscoff.fr

GR: guillaume.raulland@gmail.com

SS: sarah.samadi@mnhn.fr

CL: clejeusne@sb-roscoff.fr

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Abstract

Urban structures like marinas are dominant features of our coasts, often hotspots for invasive species. The processes that govern the distribution of invasive species within and between marinas is not well understood. We therefore investigated the impacts of local-scale variability within and between marinas, analysing fouling communities at two zones (inner and outer) within three close marinas in accordance with pollutants recorded in the water and sediment. Communities varied between zones, however no significant differences in abundances of invasive species was recorded. The inner zones contained higher levels of copper and other pollutants and were correlated with lower biodiversity and abundances of many species in comparison to the outer zones. Only the native *Ascidella aspersa* was found in greater abundances in the inner zones. This local-scale variability and how it impacts biodiversity is important for consideration for coastal managers in mitigating the build-up of pollutants and spread of invasive species.

Highlights

- ✓ Community surveys were performed analysing within and between marina variability
- ✓ A combined approach using macromorphology, microscopy and molecular methods
- ✓ Fouling communities significantly differ between inner and outer zones of marinas
- ✓ Diversity was adversely affected by higher quantities of pollutants in inner zones
- ✓ Local-scale variability is important for structuring marine fouling communities

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Introduction

As a result of the urbanization of the coastal environment, artificial structures are beginning to dominate shorelines. In Europe, Asia, the USA and Australia as much as 50% of the shoreline is now modified by hard engineering (Dafforn et al., 2015). These structures are very diverse in their shape, size and material (natural or human-made material like concrete, plastic, metals). Their function is also very diverse: coastal defences, breakwaters to provide shelter for port developments, pillars, buoys, and floating pontoons for recreational vessels, artificial islands, offshore platforms for energy extraction or renewable energy devices, and fish cages for aquaculture (Mineur et al., 2012). Furthermore, increased population densities around coastal areas have led to an increase in recreational activities such as sailing, diving and fishing (Kennish, 2002); human-made coastal defenses and marinas are built as safe havens for these vessels, often these can be sources of pollution, with vessels coated in anti-fouling paints and utilizing diesel engines. As such, these areas can be highly stressed and contaminated environments (Schiff et al., 2007).

Common contaminants in marinas are derived from boating activities; fuel spills, motor exhaust and lubricating oils are a source of hydrocarbons into marinas (Voudrais and Smith, 1986). Heavy metals, pesticides and excess nutrients, along with other pollutants, can often accumulate within marinas, originating from numerous sources such as sewage discharge, industrial activities, agricultural leaching and urban runoff (Bryan, 1971; Kennish, 2002; Rivero et al., 2013; Schiff et al., 2007). In addition, water quality is further reduced by increased suspended sediments (Iannuzzi et al., 1996) causing increased sedimentation and reduced light penetration (Airoldi, 2003). Pollutant stress in marinas is exacerbated by increased water retention caused by fixed structures (sea walls, narrow outlets) which limit circulation and mixing with adjacent waters (Floerl and Inglis, 2003). Through reduced flushing, contaminants and sediments are prevented from being dispersed and are concentrated within these environments, lowering water quality and increasing stress from chemical and physical disturbances (Rivero et al., 2013; Schiff et al., 2007).

Some of the most widespread and significant contaminants within marinas are biocides (Dafforn et al., 2008). In recreational boat harbours such as marinas, the presence of biocides is commonly greater than that found in other marine habitats (Hall and Anderson, 1999). Biocides are introduced into the water column after being applied during antifouling processes, and through the use of antifouling paints and cleaning procedures (Matthiessen et al., 1999; Schiff et al., 2007). In the past, the use of tributyltin (TBT) as a biocide caused major impacts to systems (e.g. imposex and intersex on some marine gastropods like the dogwhelks and crustaceans) and can still be detected in some water columns and sediments more than 30 years after its banishment (Dafforn et al., 2011). Today, heavy metals, such as copper, are primarily used as a basis for antifouling paints and, while necessary for growth and development of marine organisms, in high quantities they are of ecological concern (Bryan, 1971; Matthiessen et al., 1999). Metals are likely to accumulate within the tissues of organisms, and higher concentrations result in a greater impact (Johnston et al., 2011). Our knowledge of the effects of heavy metals on benthic assemblages is still limited, however typically heavy metals reduce biodiversity and abundances and facilitate the presence of opportunistic species including invasive species (Johnston and Roberts, 2009; Mayer-Pinto et al., 2015, 2010).

While the effects of contaminants will depend upon the species, season and environmental context such as habitat type (Becherucci et al., 2016; Crooks et al., 2011; Romano et al., 2010), the implications are that species tolerant to these perturbations will be found in impacted areas. Indeed, communities within marinas tend to be significantly different from that of more favorable (in terms

of water quality) habitats adjacent to impacted areas (Gittenberger and van der Stelt, 2011; Rivero et al., 2013). Furthermore, limited planktonic dispersal due to increased water retention within these locations increases propagule pressure resulting in heavier fouling than in adjacent areas (Floerl and Inglis, 2003), therefore increasing the likelihood of settlement of tolerant species. These areas are also hotspots for non-indigenous species (NIS) (Dafforn et al., 2012; Ferrario et al., 2017; Simkanin et al., 2012) introduced through maritime activities such as international and local/regional shipping, both recreational and commercial (Clarke Murray et al., 2011; Ferrario et al., 2017; Hewitt et al., 2009). NIS might tend to become invasive because they have a broader tolerance to a wider range of environmental conditions than the native species (Lejeune et al., 2014; Lenz et al., 2011; Marie et al., 2017). Additionally, the presence of human-made physical features (sea wall or settlement substrate, fixed and floating structures) often favour the settlement of NIS through relieving competitive stress by providing a novel habitat or through the alteration of circulation patterns (Airoldi et al., 2015; Foster et al., 2016; Tyrrell and Byers, 2007). These factors combine within impacted areas like marinas where common antifouling practices tend to exacerbate the problem, sometimes having a negative impact on indigenous species alone (Crooks et al., 2011), thus resulting in interactive effects that often favour NIS (Johnston et al., 2011; Piola and Johnston, 2008).

Within the marine environment, effects of a pollution gradient directly related to water quality is often observed and can have significant implications for biological communities (Je et al., 2004; Johnston and Roberts, 2009; Piola and Johnston, 2008). As such, clear differences in communities are often observed between marinas and directly adjacent areas (e.g. Rivero et al., 2013), but less is known on the localized differences and variability of communities and pollutants within marinas. The suggestion is that if contaminants are concentrating within particular sections of marinas and affecting communities, the impacts of water quality not only between marinas and adjacent systems (e.g. Rivero et al., 2013), but also within marinas themselves should become an important management consideration including for some normative certification (e.g. ISO norms, Blue Flag). It was therefore hypothesized that there would be local scale heterogeneity in communities within marinas; the areas within the innermost sections (i.e. the most confined areas, generally furthest from the entrance) would be the most heavily impacted by pollutants, possibly due to limitations on flushing with external waters, and showing clear differences such as species diversity depletion compared with the outermost sections. Furthermore, as hotspots for NIS, understanding the mechanisms behind which facilitate invasive species within marinas is imperative. If the impacts of pollutants are significant within particular locations then it follows that these areas would facilitate the growth of the most tolerant organisms such as invasive species, therefore we hypothesize that NIS would be more prevalent within the innermost locations. Monitoring suspected hotspots that can facilitate the arrival or presence of invasive species, such as at different locations within marinas, can aid ecosystem managers to help prevent and mitigate their spread (Olenin et al., 2011). Here we describe a novel approach to the traditional survey, incorporating the use macromorphological, microscopic and molecular techniques to maximize taxonomic resolution and accuracy of our survey. While molecular techniques have often been used to successfully identify the establishment of a particular species or within a particular phyla to analyze which species are present within a given location (e.g. Bishop et al., 2013; Lopez-Legentil et al., 2015), there are fewer examples using all three methodologies to verify and maximize the accuracy within taxonomic surveys. Compared with traditional surveys of benthic communities, incorporating molecular techniques to characterize biodiversity is important step in an environment where cryptogenic and introduced species are common and often unidentified using morphological identification alone.

Methodology

Study site

The three study sites were closely located within the Bay of Brest, France. Brest is a large port city with a history of industrial activity related to maritime craft, including both commercial and military activities. Within the city, there are two recreational ports: the Château Marina (CHA; 48°22'44"N, 4°29'21.0"W), and the Moulin Blanc Marina (48°23'31"N, 4°25'54"W). Moulin Blanc Marina is made up of two distinct marinas, built at different times (1961 and 1980) and separated by a seawall, these will henceforth be referred to as Moulin Blanc North (MBN) and Moulin Blanc South (MBS). CHA is situated at the entrance to the Penfield River in close proximity to the commercial and military ports. MBN and MBS are located close to the larger Elorn River. Combined, the Moulin Blanc Marina has 1460 berths, a slipway in both MBN and MBS, and incorporates a fueling station and a recreative shipyard in MBN. By comparison CHA incorporates 625 berths and a fueling station, but has neither slipways nor naval activities (Figure 1).

Within each site, two study zones were identified according to the distance from the entrance of the marina (Figure 1), situated in the innermost and outermost sections of the marinas (referred to as the inner and outer zones). Within each zone, two metal pillars (1m diameter) were selected for sampling. Sampling took place in late February and early March 2016. Samples were collected from each site within the same day.

Sampling procedures

Sampling was conducted by SCUBA diving whereby each quadrat was located 50 cm below chart datum. Three quadrats (0.2 m x 0.25 m) were randomly placed on each pillar, totalling six within each zone and twelve per marina. Quadrats were photographed and organisms were subsequently scraped into 1 mm meshed nets. Nets were transported to Roscoff Biological Station in aerated seawater taken from the marinas and subsequently placed within aerated aquaria prior to identification. We sorted the animals by eye, identifying to the lowest taxonomic level we could. Individuals and colonies that we were not able to identify by eye were fixed within 100% ethanol to characterize the specimens using either additional morphological characteristics under the microscope or, with molecular characteristics. While we were unable to ascertain species level identification for all specimens, using both methodologies this combined approach allowed us to identify specimens to as fine a taxonomic resolution as possible. Species were classified invasive based upon published biogeographic and genetic data supporting a non-native origin (*sensu* Airoidi et al., 2015). All individuals and colonies were quantified according to the MNCR SACFOR abundance scale (Connor et al., 2004). The SACFOR scale allows colonial and individual organisms to be quantified on the same scale (0–6) according to their relative abundances and sizes, where 1 = rare and 6 = superabundant. A value of 0 is given when species are absent. Due to an error, one quadrat was missing from within the inner zone of MBN, totalling five replicates for this zone. Mobile fauna (decapods, polychaetes and echinoderms) and algae were noted, however these were removed from the subsequent analyses, which were conducted on the sessile faunal community only.

DNA barcoding

DNA barcoding was used as a complementary tool to identify the collected specimens. DNA was extracted using the NucleoSpin® Tissue 96 according to the standard protocol kit. Recovered DNA was amplified for the Cytochrome oxidase 1 (Cox1 or COI) barcoding marker through 3-step PCRs, whose conditions and primers differed depending on the amplified phylum (Supplemental

Material). For each taxon, the amplification reaction was carried out in a volume of 25 μ L, containing 0.2 mM dNTP, 1.8 mM of $MgCl_2$, 0.6 μ M of each primer, 1 x buffer and 0.5 unit of GoTaq[®] Flexi from Promega. The PCR products were checked on 1% agarose gel then sequenced by the Eurofins Genomics Company. Sequences were edited and aligned using the software CodonCodeAligner 5.1.5. These sequences were then used in phylogenetic reconstructions by incorporating sequences selected in BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/GenBank). The accuracy of identification relies on the taxonomic diversity covered by sequences available in databases but also on the confidence we may have in the associated taxonomic identification. We thus selected the sequences that are, as much as possible, best associated with voucher specimens. Indeed, if unexpected results are obtained, voucher specimens might be examined to reevaluate the identification. Phylogenetic reconstruction was carried out using the software MEGA6 (version 6.06). Phylogenetic reconstruction were conducted using the Neighbor-Joining (NJ) method on p-distances. The robustness of the reconstruction was tested using bootstrapping with 1000 replicates.

Pollutant sampling

Water and sediment samples were collected from each zone and site. One sample was collected from each zone resulting in six water and bottom sediment samples in total. Samples were collected in between the two pillars sampled for macrofauna, with water samples collected at the same depth as the macrofauna. Sediment was collected because it was expected that pollutants would accumulate within the sediment and be representative of the conditions within the marinas over a longer period of time than the water samples which could be expected to change temporally (Bryan and Langston, 1992; Rainbow, 1995), for example due to daily flushing and tidal regimes. Samples were processed for pollutants, pesticides and heavy metals by the EUROFINS Environment Company (see supplementary material for the complete list of contaminants tested for).

Data analysis

Permutational multivariate or univariate analysis of variance (Anderson et al., 2008) was utilized to analyze differences between sites and zones. Three factors were included to take into account the sampling design whereby three replicates were taken from each of two pillars within each zone (total $n = 6$, except for inner MBN where $n = 5$). As such, zone (two levels: Inner and Outer) and site (three levels: CHA, MBN and MBS) were designated fixed factors. Site was designated as a fixed factor due to our objective to compare the differences between the sites. To take into account any variation between pillars, pillar was designated a random factor (two levels) nested within site and zone. Where differences between factors occurred, PERMANOVA *posteriori* pairwise comparisons were conducted and analyzed using Monte Carlo testing. For multivariate analyses, SACFOR abundances were used without transformation and a Bray-Curtis similarity matrix was constructed. Multivariate community data was visualized using a principal coordinates analysis (PCoA). For univariate data (specific taxa and species richness), analyses were conducted on Euclidean distance resemblance matrices. SIMPER analysis was used to discriminate which species were responsible for differences between factors, defined as those having a dissimilarity/standard deviation ratio > 1 . Canonical analysis of principal coordinates (CAP; Anderson and Willis, 2003) was used as a constrained ordination to visualise the correlations between pollutant variables and the multivariate macrofauna data. All analyses were conducted using PRIMER v6 and PERMANOVA+ add on (Anderson et al., 2008; Clarke and Gorley, 2006).

Results

In total, 58 taxa were identified (mostly to species or genus level) across all sites; 42 were found at CHA, 24 at MBS, and 37 at MBN. Assemblages were made up of encrusting and arborescent bryozoans, colonial and solitary ascidians, barnacles, bivalves, hydrozoans, and serpulid and sabelid polychaetes (Table 1). Of these, nine were considered invasive, although only three were found in more than three quadrats: *Watersipora subatra*, *Didemnum* sp. and *Asterocarpa humilis*. A total of 15 out of 20 taxa checked for identification through DNA barcoding were successfully amplified and sequenced (Table 1). DNA barcoding confirmed the identities of these taxa at equivalent resolution compared with standard macro-morphological and microscopic techniques (Table 1), however, it helped in solving some ambiguities due to taxonomically similar species with unlisted or uncommon polymorphism (color, form; e.g. *Obelia dichotoma*, *Tricellaria inopinata*), in identifying small specimens lacking standard features (e.g. *Ascidrella scabra*, *Morchellium argus*), and for identification of invasive species (e.g. *Perophora japonica*).

The community structure and diversity significantly varied between site and zone (Table 2). While there was a significant interaction between factors in the multivariate community analysis (Table 2), there was clear separation between zones (Figure 2). Pairwise tests revealed that while CHA displayed clear differences between the inner and outer zones ($t = 2.91$, $p(\text{MC}) = 0.009$), differences between zones within MBS ($t = 1.78$, $p(\text{MC}) = 0.086$) and MBN ($t = 1.61$, $p(\text{MC}) = 0.082$) were less distinct. Pairwise testing also revealed that there were significant differences between the outer zones of CHA in comparison to both MBS ($t = 2.53$, $p(\text{MC}) = 0.009$) and to MBN ($t = 2.02$, $p(\text{MC}) = 0.029$), but not between outer zones of MBS and MBN ($t = 1.11$, $p(\text{MC}) = 0.341$). The inner zones of all three sites did not display any significant differences (pairwise comparisons $p > 0.05$).

PCO vectors (Figure 2) with a Pearson correlation > 0.5 reveal the differences between zones are driven by the presence of *Ascidrella aspersa* in the inner zones and a greater abundance of numerous species in the outer zones. Furthermore, *Oscarella rubra*, *Perforatus perforatus*, *W. subatra*, *M. argus*, *Hiatella arctica* and *A. scabra* are strongly correlated in the outer zones, particularly within CHA.

Strong differences between zones are further evidenced by the lower species richness observed in the inner zones at all three sites (Table 2; Figure 3). Pillar was observed to have a significant effect on species richness however this is due to greater difference between the two pillars in the outer zone of MBN in comparison to the other sites and zones, pairwise comparisons reveal these differences were not significant ($p > 0.05$). No differences in species richness of invasive species were observed between zones or sites, although MBS quadrats typically contained fewer invasive species (Table 1).

A. aspersa, *P. mammillata*, *Didemnum* sp., *Balanus* spp. and the anomidae were among the most abundant and ubiquitous species found in all sampling locations. These species were identified in the SIMPER analysis along with *A. scabra*, *M. argus*, *Amathia pustulosa* and *W. subatra* as those contributing to differences between zones and sites (Figure 4). Of these, *Didemnum* sp., *P. mammillata*, *Balanus* spp. and the anomidae did not display any significant differences between sites or zones ($p > 0.05$). Two species displayed a significant main effect of zone (PERMANOVA $p < 0.05$) whereby the abundance of *A. aspersa* was significantly greater in the inner zone and *A. pustulosa* was greatest in the outer. *W. subatra* displayed significant differences between sites only and no effect of zone. Significant interactions between site and zone (PERMANOVA $p < 0.05$) were observed for *A. scabra* and *M. argus*. Both species displayed greater abundances in the outer zones in CHA,

additionally *M. argus* displayed greater abundances in the outer zone of MBN. There were no differences in abundances between zones for either species in MBS. *A. scabra* was not observed in MBN.

The pollutant analysis revealed that within the marinas, most of the pollutants and pesticides tested for were below the detection limit (see supplementary material for the complete list). Within the water column and at all sites, only zinc, hydrocarbons, nitrates and orthophosphates were present (Table 3a). Within the sediments, zinc, copper and hydrocarbons were identified in high quantities (Table 3b). Of the pesticides discovered, ametryn, atrazine, prometryn, propazine and simazine were only present within the sediments of the outer region in MBS, whereas organochlorides such as DDT and β -HCH were found in the sediments within the outer regions of MBN, with lindane additionally present in the sediments of both MBN and MBS (Table 3b). CAP analysis evidenced strong associations between the macrofaunal community structure and the environmental variables (Figure 5). While there was clear separation between inner and outer zones in the ordination constrained by the water pollutants (Figure 5a), there were weak correlations between individual pollutants and the community structure, however the nitrates and total hydrocarbons had a clear impact in differentiating between sites and were found in higher quantities in MBN and MBS. The analysis constrained by the sediment pollutant data (Figure 5b) provided strong evidence to suggest that the copper (and to a smaller extent hydrocarbons) found in higher quantities in the inner zones, had a large effect on differentiating communities between zones where copper was discovered in greater quantities within the inner zones (Table 3b). Furthermore, communities appear to be strongly influenced by zinc and the organochloride pesticides in structuring between sites, with zinc found in higher quantities in CHA, and the organochlorides in higher quantities in MBN and MBS (Table 3b).

Discussion

This study highlights the potential for variation to exist within marinas; clear differences in community structure were identified between zones within the marinas in Brest which could be linked to the presence of pollutants. Marinas are known to modify the biota of sessile communities in comparison to adjacent environments (Rivero et al., 2013) by altering the environmental dynamics and flow patterns (Floerl and Inglis, 2003). Given that the structure of marinas facilitate the formation of eddies, enhancing water retention (Floerl and Inglis, 2003), the innermost sections are likely to become impacted to a greater extent due to the effects of increased contaminants caused by reduced flow and limited flushing (Schiff et al., 2007). Water flow is also a key factor influencing larval settlement (Koehl, 2007); the combined consequences of reduced flows and heavy pollution will promote the settlement of the most tolerant species, and these local processes will be important for structuring biodiversity (Simpson et al., 2017). While this study did not observe any differences in the presence or the abundance of invasive species between the innermost and outermost zones of the marinas, there was a clear difference in community structure and biodiversity between zones.

Through the use of multiple identification techniques (macro-morphological identification, microscopy, and DNA barcoding) we were able to conduct surveys maximising taxonomic resolution. While many studies report using a particular technique for identification purposes, few studies report using DNA barcoding explicitly to complement standard techniques. DNA barcoding is an efficient tool notably to detect invasive species because morphological identification keys are often lacking or are built for identifying organisms at a regional scale (e.g. Hayward and Ryland, 2017). This technique helps to confirm morphological identification when identifying features are lacking or when specimens are small (Bucklin et al., 2011). We successfully used barcoding to complement standard

microscopy techniques for detecting invasive species where we were unsure of the identity (e.g. *Perophora japonica*) and for confirming the identity of similar species. We believe these efforts should be conducted in ecological surveys to fully characterise biodiversity, particularly in cases where taxonomic expertise is lacking and species identities can be easily mismatched.

Flow patterns within marinas are mediated by the types of structure present, and regimes differ depending on the location within the marina (Floerl and Inglis, 2003). Being close to the edge of a marina would facilitate maximal water exchange with adjacent areas, thus reducing the levels of pollutants within the water column and facilitating exchange of propagules. Community structure is often regulated by environmental cues, both chemical and physical (Menge and Olson, 1990). While in this study there were differences between sites, the greatest variation was observed between zones within the marinas. Interestingly, there were no observable differences in community structure among the inner zones of all three marinas. One explanation could be that reduced flow patterns led to decreased settlement within the innermost zones (Oricchio et al., 2016), facilitating the observed differences. However many authors have found evidence to suggest that early recruitment does not always explain later stages of community composition (e.g. Bram et al., 2005; Mook, 1981; Sams and Keough, 2013), particularly when propagules from all species are present and spread around the marina. Given that we analysed established communities and that levels of pollutants such as copper and hydrocarbons were greater within the inner regions, we expect that these factors, in combination with hydrodynamic processes, are more likely to explain the observed differences.

Lowered diversity is often a consequence of increased pollutants (Johnston and Roberts, 2009) and reductions in diversity are often observed in similar polluted environments (e.g. boat harbours; Je et al., 2004). Of the pollutants recorded in this study, community structure between zones is most strongly correlated with the presence of copper. Often used as a biocide, copper not only acts to reduce biodiversity, but can also alter ecosystem functions provided by the community (Canning-Clode et al., 2011; Kinsella and Crowe, 2016). Furthermore, the effect of hydrocarbons within the water column is likely to contribute to further detrimental effects on marine organisms (Dauvin et al., 2012; Johnston et al., 2015) and potentially interact synergistically in combination with heavy metals such as copper (Cebrian and Uriz, 2007).

Within this study, other pollutants were identified as being strongly correlated to community structure. Zinc, also used in antifouling paints, is known to have harmful effects on marine communities (Matthiessen et al., 1999; Ytreberg et al., 2010). There is also the potential for this pollutant to display interactive effects with other metals (e.g. lead; Mahmoudi et al., 2007). In this study, the presence of zinc was related to the community differences among marinas, however levels recorded within the water column were not great enough to illicit detrimental effects due to chronic exposure observed in other studies (Matthiessen et al., 1999). Nitrates and orthophosphates were observed, however they appeared to have a limited influence on structuring communities. While important for structuring and facilitating changes in communities in areas of nutrient limitation or in other marine environments (Johnston and Roberts, 2009; Kenworthy et al., 2016; Mayer-Pinto and Junqueira, 2003), on hard substrates, the availability of space is of greater importance as a key limiting resource (Dayton, 1971; Stachowicz et al., 1999). Increased nutrients causing eutrophic conditions are known to promote algal production, particularly within the phytoplankton (Cloern, 2001). This, in turn, could facilitate changes of the filter feeding organisms present within fouling communities, however these communities also act to control the phytoplankton blooms through feeding activity, thus limiting the impact (e.g. Hily, 1991). While not our aim, within this study, we

noted that there was a larger presence of primary producers found in MBN, however the abundance of primary producers found on the pillars was, overall, extremely low. Furthermore the presence of nutrient pollution was only recorded within the water column, which had weaker correlations due to the fact that these are ephemeral and replaced due to passage of tides (see methods) and may not be representative of the daily impacts found in these locations.

While present within this study, most NIS only occurred in a small number of quadrats in relatively low abundances compared to indigenous species. Surprisingly, and contrary to our predictions, NIS were not found to be more common within the inner parts of the marinas. There are many experimental examples of where pollutants such as copper impacts native species and facilitates the prevalence of NIS (Canning-Clode et al., 2011; Crooks et al., 2011; Piola and Johnston, 2008). Higher concentrations of pollutants can facilitate recruitment of NIS due to lower pressure from native species (Crooks et al., 2011; Foster et al., 2016; Johnston et al., 2011) and a supposed higher tolerance to pollutants (Karatayev et al., 2009; Piola and Johnston, 2008), so it was assumed the inner zones could be the most likely place for NIS communities to be established. Lack of differences may be attributed to seasonal die off associated with winter months however further studies of seasonal patterns need to be conducted to confirm this. For example, within marinas in Brittany, the relative abundance of the invasive *Ciona robusta* to its native congener *C. intestinalis*, increased in the autumn compared with spring (Bouchemousse et al., 2016). Long-term studies of seasonal variation need to be conducted in order to fully understand the impact of invasive species.

While NIS were found in low abundances, approximately 15% of the total number species identified were classified as invasive. In particular, *Didemnum* sp., a species known to proliferate in marinas and other artificial structures (Simkanin et al., 2012), was ubiquitously identified at all sites and zones. Other invasive species were also identified in a large number of quadrats. *A. humilis* was also identified at all three sites, this species has been overlooked and misidentified within NW Europe and is only recently recognised as a widespread and common invader, especially within marinas (Bishop et al., 2013). *W. subatra* was found in greatest abundance in the outer zone of CHA and is known as a prolific coloniser in artificial habitats, particularly within Brittany (Bishop et al., 2014). The high presence of these species and other NIS in our study exemplifies patterns observed by others. Within marinas and other artificial substrates, NIS are known to be particularly abundant and pervasive, especially in comparison to natural habitats (Dafforn et al., 2012; Simkanin et al., 2012). Higher tolerances to a range of environmental conditions often facilitate the presence of these species (Crooks et al., 2011; Lenz et al., 2011), and, once established within a marina, NIS can quickly spread (Bishop et al., 2014) due to faster metabolic rates causing rapid growth and short generation times compared with native species (Lagos et al., 2017). It has been suggested that tolerance in invasive species is a heritable trait (e.g. McKenzie et al., 2011). The possibility therefore exists for species to become adapted to the higher contaminant levels located within certain sections of marinas, which in turn could facilitate and enhance the spread of the more tolerant genotypes, either through spread to adjacent habitats or through transport on fouled vessels. Understanding the tolerance of potential invaders is therefore paramount in ecological research, with such studies helping to reduce and manage their spread.

Within the present study, the reduced biodiversity and differing community compositions between zones is indicative of the varied tolerances and sensitivities exhibited by different species (Canning-Clode et al., 2011; Luoma and Rainbow, 2005). Most strikingly, within this study *Ascidella aspersa* was the only species to be found in greatest abundance within the inner zones of all three marinas.

While copper levels in sufficiently high quantities in the water column have been known to have a negative effect on this species (e.g. Johnston and Keough, 2003), copper based antifouling paints have been shown to have no effect on this species (Braithwaite et al., 2007). Furthermore, *A. aspersa*, classified as a NIS outside of Europe, is a highly tolerant species utilizing effective reproductive strategies that promote settlement year round and respond rapidly to changes in environmental conditions (Lynch et al., 2016). *Amathia pustulosa* was the only other species to display a zone effect, showing a preference for outer zones. Many other species displayed site specific responses, showing preferences for the outer zones. These differences were greatest within CHA, which, of the three marinas studied, was the most restricted, enclosed by a breakwater and narrow entrance (C. L., pers. comm.), thus limiting flows and circulation within the marina (Floerl and Inglis, 2003). It was noted that Porifera such as Halichondriidae and *Oscarella rubra* were present in greater abundances at CHA. Both groups were rare, or absent in the case of Halichondriidae, in the inner zones of CHA but common in the outer zones. In other locations, greater settlement of sponges inside marinas in comparison to equivalent environments adjacent has been recorded (Rivero et al., 2013; Turner et al., 1997), and in greater abundance in more enclosed marinas (Floerl and Inglis, 2003) such as CHA. Filter feeders such as sponges are sensitive to sedimentation (Airoldi, 2003), which is often greater within the inner parts of marinas. While likely to cause an impact on species, it has been noted that filter feeders can still prevail within high sedimentation environments such as marinas (Rivero et al., 2013), and even within our study, certain species, like *Suberites ficus* in MBS, were only observed within the inner regions.

Within our study, we focussed solely on communities on a single substrate: metallic pillars. Within marinas, varied types of substrate can be found: floating plastic pontoons, metallic and wooden pillars, and concrete structures. Substrate type is known to have an effect on the distribution of organisms (Chapman and Clynick, 2006), and different species, including NIS, can show a preference for a certain type of substratum (Chase et al., 2016; Gittenberger and van der Stelt, 2011). For example, Chase et al. (2016) discovered a preference for the invasive species *Botrylloides violaceus* to settle on a concrete substrate over granite, HDPE and PVC, whereas an alternate preference was observed for the native *Ciona intestinalis*. The authors argue that these preferences were due to substrate surface roughness and chemicals leaching from the material. In addition to the substrate type, the substrate orientation is also a major determinant affecting community structure and NIS (Dafforn et al., 2012; Gittenberger and van der Stelt, 2011). For example, in a study by Gittenberger and van der Stelt (2011) in the North Sea, *A. aspersa* showed a preference to underhanging habitats as opposed to vertical surfaces. Our results suggest that on vertical metallic structures, while significant differences in communities correspond to the differences in pollutants within these marinas, NIS do not show the same effect. Further research is needed to ascertain whether this relationship holds true for other substrate types.

Conclusion

As the coastal environment is becoming more urbanised, structures are being built to facilitate coastal activities. Marinas are common urbanised features housing artificial structures such as pilings, pillars and pontoons and they are known to cause vast differences in communities in comparison to natural environments, and facilitate invasive species. This study identified that this structuring effect can occur between locations within a marina and that local scale variability is an important aspect in determining community structure within and between marinas located within the same geographic region. These areas alter water flow patterns and are sources of pollution, however small scale

differences can also be present within marinas, to the detriment of communities. Differences in pollutants were correlated with the structure of sessile communities within and among marinas. Lower biodiversity was observed within the innermost zones, where pollutant tolerant species were observed to prevail. While not directly observed in this study, this has the potential to exacerbate the problem of invasive species, facilitating their settlement within particular zones of a marina. In addition, there is potential for these higher contaminant zones to select for the most tolerant individuals which in turn could enhance the spread of these species. This is a particularly important for consideration for ecosystem managers to reduce the spread of invasive species. To fully explore the effects of this local variability within marinas, future explorations should be investigated at different times of year, carefully considering the role of pollution in structuring these communities, where heavily impacted sites will likely become colonised by the most tolerant (and potentially invasive) species. This highlights the necessity to evaluate which factors, such as pollutants, are most important for structuring sessile marine communities. Impacts to water quality should be considered when designing marinas to prevent and manage the build-up of polluted areas which can have significant consequences to environmental quality and enhancing the spread of invasive species.

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Table 1: Number of occurrences of species from within quadrats in the outer and inner regions of 3 marinas in Brest, France (Chateau: CHA; Moulin Blanc North: MBN; Moulin Blanc South: MBS). Identification criteria signifies the method that obtained the finest taxonomic resolution: Mor = Macromorphology, Mic = Microscopy, DNA = successful DNA barcoding. fDNA = unsuccessful DNA barcoding. Non indigenous species are identified (NIS). n = 6 quadrats per region except MBN where n = 5.

Species	Identification criteria	Status	Outer			Inner		
			CHA	MBN	MBS	CHA	MBN	MBS
Annelida								
<i>Branchiomma bombyx</i>	Mor		1	0	0	0	0	0
<i>Sabella pavonina</i>	Mor		0	1	0	1	0	0
<i>Serpulidae</i>	Mor		0	2	0	0	0	0
Arthropoda								
<i>Austrominius modestus</i>	Mor, Mic	NIS	0	0	0	2	0	0
<i>Balanus</i> spp.	Mor, Mic		1	5	6	2	3	2
<i>Perforatus perforatus</i>	Mor		4	1	0	1	2	0
Bryozoa								
<i>Amathia gracilis</i>	Mic		0	1	0	0	0	0
<i>Amathia pustulosa</i>	Mic, DNA		1	4	4	0	1	2
<i>Bugulina flabellata</i>	Mic		0	1	0	0	0	0
<i>Callopora rylandi</i>	Mic, fDNA		0	0	3	0	0	0
<i>Scruparia chelata</i>	Mic		0	1	0	0	0	0
<i>Scrupocellaria scruposa</i>	Mic		3	5	2	0	1	0
<i>Tricellaria inopinata</i>	Mic, DNA	NIS	1	1	0	0	0	0
<i>Tubulipora</i> sp.	Mic		0	0	0	1	0	0
<i>Vesicularia spinosa</i>	Mic		0	4	0	1	1	0
<i>Watersipora subatra</i>	Mic, DNA	NIS	6	2	0	2	2	0
Chordata								
<i>Aplidium elegans</i>	Mic		1	0	0	0	0	0
<i>Aplidium proliferum</i>	Mic, fDNA		0	1	0	1	0	0
<i>Ascidia mentula</i>	Mor		0	0	0	5	0	1
<i>Ascidiella aspersa</i>	Mor, DNA		0	3	4	6	5	6
<i>Ascidiella scabra</i>	Mor, DNA		6	0	1	2	0	0
<i>Asterocarpa humilis</i>	Mor, fDNA	NIS	1	0	1	3	1	0
<i>Botrylloides</i> sp.	Mor		0	2	0	0	3	0
<i>Botryllus schlosseri</i>	Mor		2	1	0	2	0	0
<i>Ciona intestinalis</i>	Mor		0	0	0	1	0	0
<i>Corella eumyota</i>	Mor	NIS	0	0	0	1	0	0
<i>Didemnum</i> sp.	Mic, DNA	NIS	5	6	5	4	5	6
<i>Microcosmus</i> sp.	DNA		0	0	0	1	0	0
<i>Molgula tubifera</i>	Mic		1	1	0	0	0	0
<i>Morchellium argus</i>	Mor, Mic, DNA		6	4	3	0	0	1
<i>Perophora japonica</i>	DNA	NIS	0	3	0	0	0	0
<i>Phallusia mammilata</i>	Mor		1	3	3	4	2	2
<i>Polycarpa pomaria</i>	Mor, DNA		0	0	0	0	1	1
<i>Pyura microcosmus</i>	Mor, Mic, fDNA		1	0	1	4	0	1
<i>Pyura squamulosa</i>	Mic		0	1	0	0	0	0
<i>Styela clava</i>	Mor	NIS	0	2	0	0	0	0
Cnidaria								

<i>Campanularia hincksii</i>	Mic		1	2	2	1	0	0
<i>Cerianthus lloydii</i>	Mic		1	0	0	0	0	0
<i>Laomedea flexuosa</i>	Mic		0	2	0	1	1	0
<i>Obelia bidentata</i>	Mic		0	0	1	0	0	0
<i>Obelia dichotoma</i>	Mic, DNA		1	2	1	0	2	1
Mollusca								
Anomidae	Mor		5	3	6	3	3	1
<i>Crepidula fornicata</i>	Mor	NIS	0	0	1	0	0	0
<i>Hiatella arctica</i>	Mor		3	0	0	0	0	0
<i>Modiolula phaseolina</i>	Mor, Mic		0	2	0	0	0	0
<i>Musculus subpictus</i>	Mor, Mic		0	0	1	2	1	0
<i>Ostrea edulis</i>	Mor		0	1	0	0	1	0
Unidentified bivalve	Mor		0	1	1	0	0	0
Porifera								
<i>Crella (Yvesia) rosea</i>	Mic		1	0	0	0	0	0
<i>Halichondria</i> spp.	Mic, DNA		2	6	0	0	0	0
<i>Haliclona</i> sp.	Mic		0	2	0	0	0	1
<i>Hymeniacidon perlevis</i>	Mic, DNA		2	0	0	0	0	1
<i>Mycale macilenta</i>	Mic, fDNA		2	0	0	0	0	0
<i>Oscarella rubra</i>	Mor, DNA		4	3	2	0	0	0
<i>Suberites ficus</i>	Mic, DNA		0	0	0	0	0	4
<i>Sycon ciliatum</i>	Mor		1	1	0	0	0	0

Table2: Three-factor PERMANOVA results examining the differences between zones (inner and outer) at 3 sites (CHA, MBS and MBN) within Brest. Pillar was nested within Z x S. N = 5–6 for each zone.

	df	Multivariate		Species Richness	
		Pseudo-F	p(perm)	Pseudo-F	p(perm)
Zone	1	8.49	0.004	7.47	0.042
Site	2	3.65	0.006	3.31	0.108
Zone x Site	2	2.39	0.025	0.23	0.818
Pillar	6	1.27	0.114	2.71	0.029
Residuals	23				

Key: **Bold** = significant effect at $p < 0.05$

Table 3: Total and average pollutants discovered within the water column (a; mg L⁻¹) and the sediments (b; mg kg⁻¹) at 2 zones (Inner: IN; Outer: OUT) within 3 marinas in Brest, France (Chateau: CHA; Moulin Blanc North: MBN; Moulin Blanc South: MBS). Quality guidelines are included (where available) outlining quality standards from various sources. These give an indication of the lower limits at which water or sediments are considered polluted and not necessarily the level at which will have an impact on organisms.

a) Water		Hydrocarbons	Zinc	Nitrates	Orthophosphates							
	MBS IN	19	0.0018	1.101	0.0418							
	MBS OUT	40	0.0019	1.111	0.0332							
	MBN IN	36	0.0069	1.463	0.0541							
	MBN OUT	30	0.0076	1.371	0.0608							
	CHA IN	19	0.0037	0.5561	0.5793							
	CHA OUT	5	0.0018	0.4235	0.0798							
	Average MBN	30	0.0019	1.1060	0.0375							
	Average MBS	33	0.0073	1.4170	0.0575							
	Average CHA	12	0.0028	0.4898	0.3296							
	Average Inner	25	0.0041	1.0400	0.2251							
	Average Outer	25	0.0038	0.9685	0.0579							
Quality guidelines		0.3 ^{a,b}	0.04 ^a	5.3 ^{a †}	0.087 ^{a †}							
b) Sediment		Hydrocarbons	Zinc	Copper	Lindane	β-HCH	DDT	Ametryn	Atrazine	Prometryn	Propazine	Simazine
	MBN IN	341	140	84	0.02	0	0	0	0	0	0	0
	MBN OUT	238	108	42	0.03	0.05	0.04	0	0	0	0	0
	MBS IN	261	162	93	0.02	0	0.01	0	0	0	0	0
	MBS OUT	174	112	59	0.17	0	0	25	95	2	9	26
	CHA IN	1320	446	93	0	0	0	0	0	0	0	0
	CHA OUT	156	175	44	0	0	0	0	0	0	0	0
	Average MBN	290	124	63	0.03	0.03	0.02	0	0	0	0	0
	Average MBS	218	137	76	0.10	0.00	0.01	13	48	1	5	13
	Average CHA	738	311	69	0.00	0.00	0.00	0	0	0	0	0
	Average Inner	641	249	90	0.01	0.00	0.00	0	0	0	0	0
	Average Outer	189	132	48	0.07	0.02	0.01	8	32	1	3	9
Quality guidelines		50-200 ^{b,c ‡}	124 ^a	18.7 ^a	0.00032 ^d	-	0.001 ^d	*	*	*	*	*

a Cole et al. 1999

b Adeniji et al. 2017

c Massoud et al. 1996

d CCME 2001

† Quality guidelines based on riverine and estuarine data, other factors can impact quality in coastal and marine environments

‡ There are no specific guidelines for petroleum based hydrocarbons within sediments but levels between 50-200 mg kg⁻¹ can be considered to be moderately polluted

* There is lack of information for these pollutants within sediments however water quality guidelines exist (see CCME 2001)

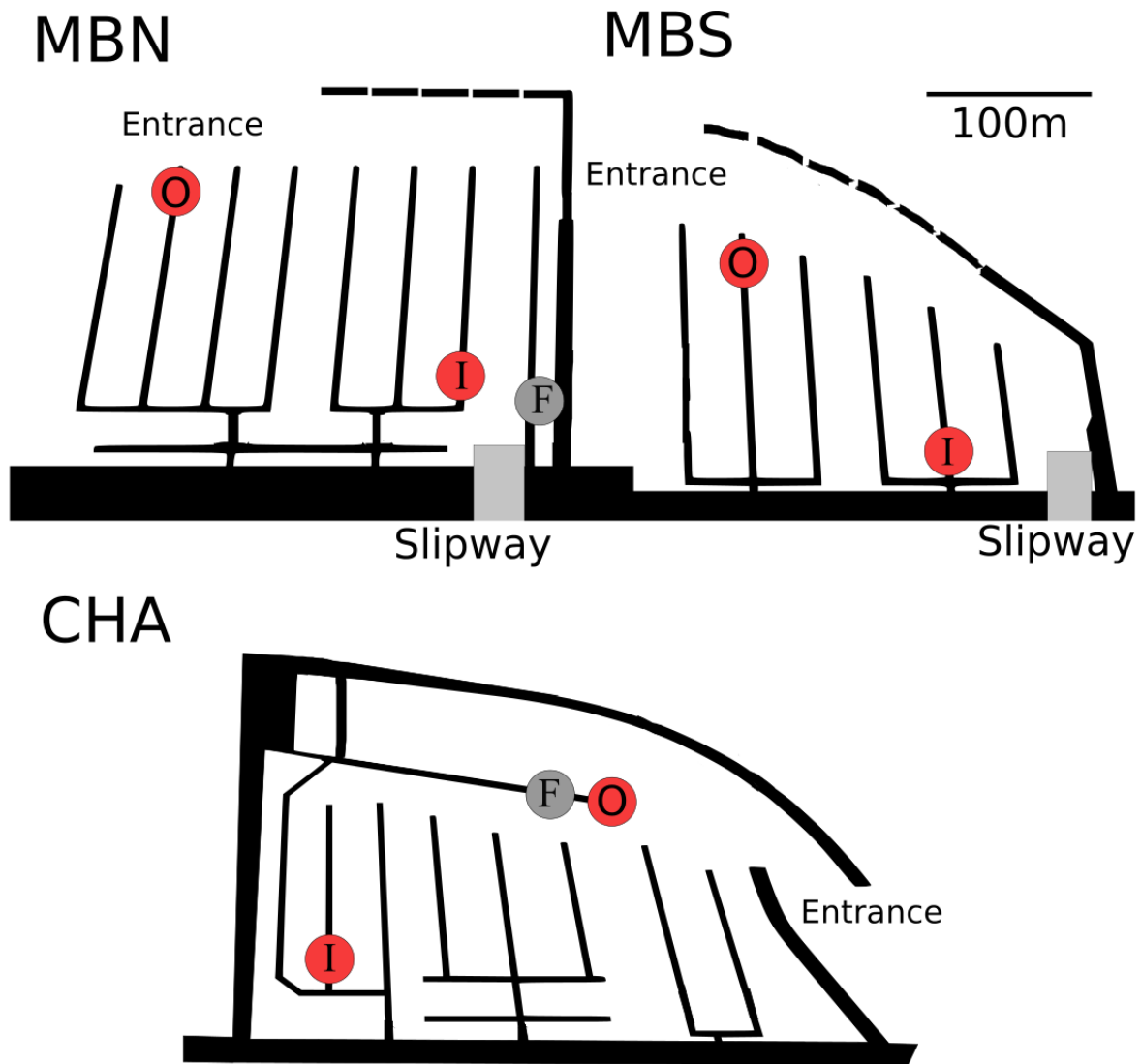


Figure 1 : Schematic representing the location of the sampling sites within 3 marinas in Brest, France (MBN = Moulin Blanc North; MBS = Moulin Blanc South; CHA = Chateau marina). Sampling sites are located in the innermost (I) and outermost (O) sections of the marinas. Slipways and fueling stations (F) are also noted.

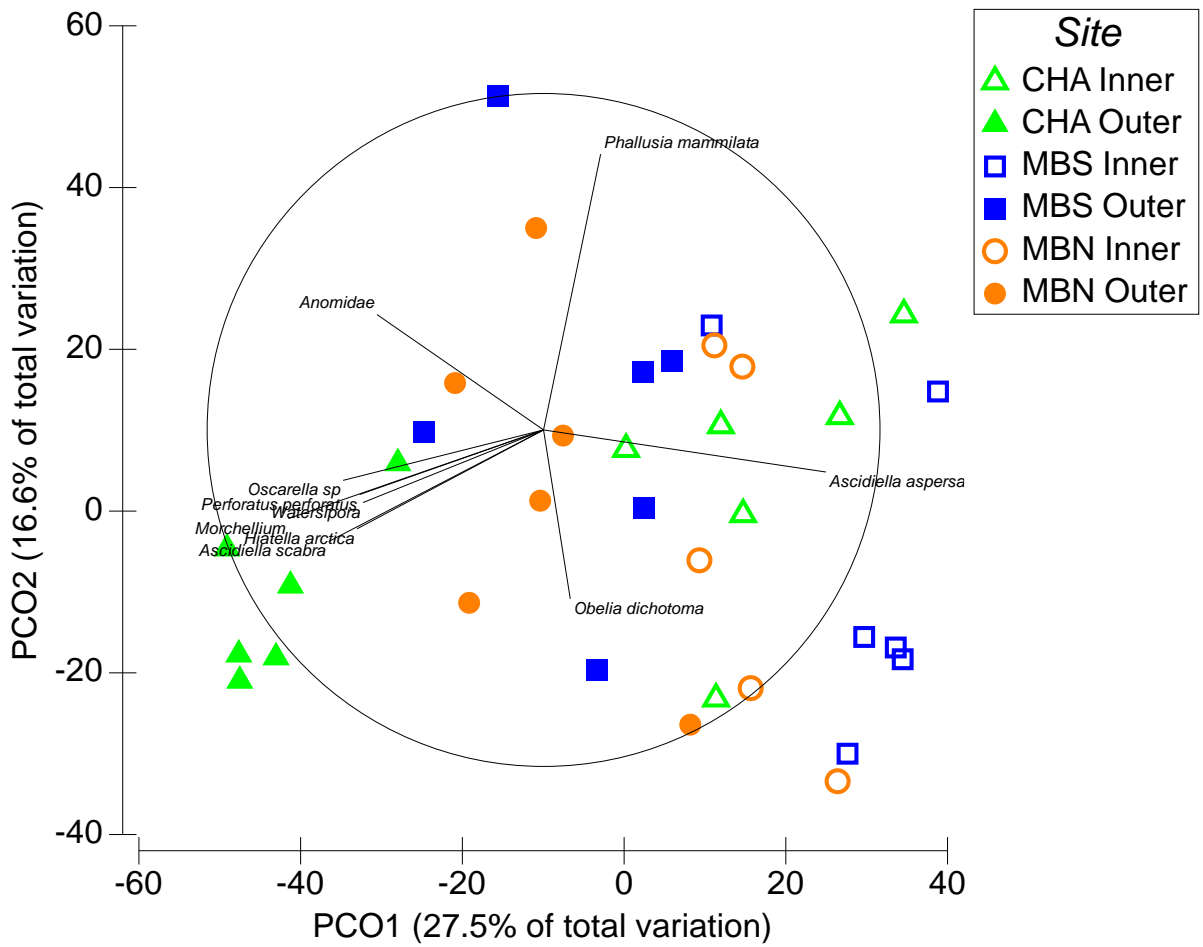


Figure 2: Principal Coordinate Analysis (PCoA) based on SACFOR abundance of macrofaunal communities at inner (INT: open symbols) and outer (EXT: closed symbols) sites in marinas in Château (CHA), Moulin Blanc North (MBN) and Moulin Blanc South (MBS). Vector overlay indicates taxa which were positively correlated (>0.5) with the axes.

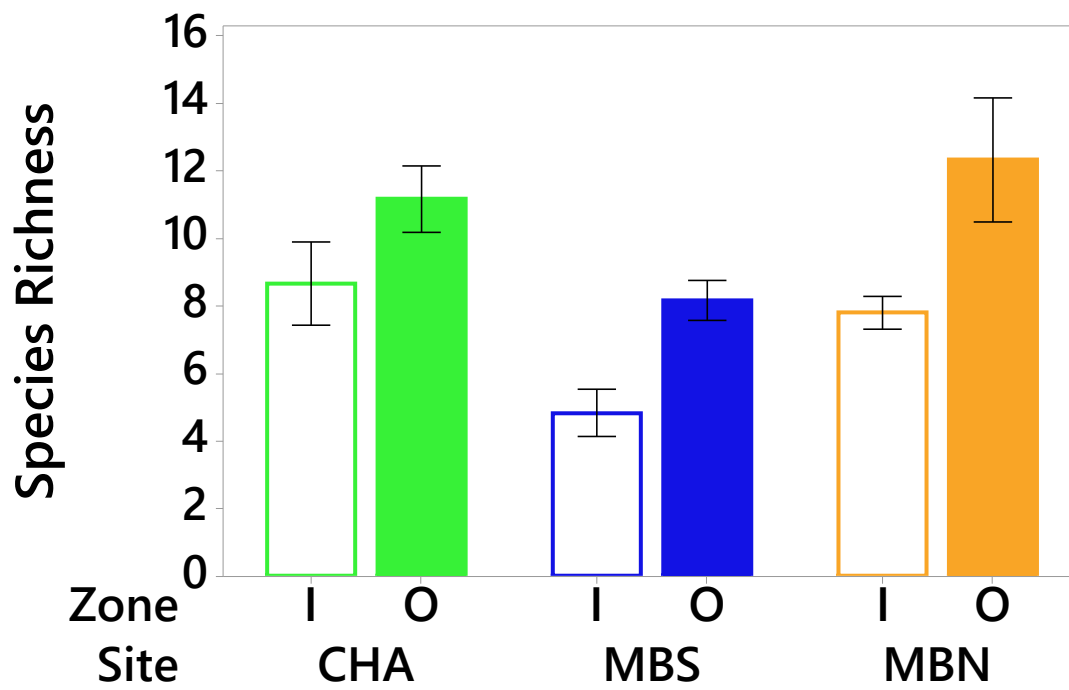


Figure 3 : Mean (\pm SE) species richness at inner (I: open bars) and outer (O: closed bars) zones of three different marinas within Brest (Château, CHA; Moulin Blanc North, MBN; Moulin Blanc South, MBS). N = 5–6 for each zone

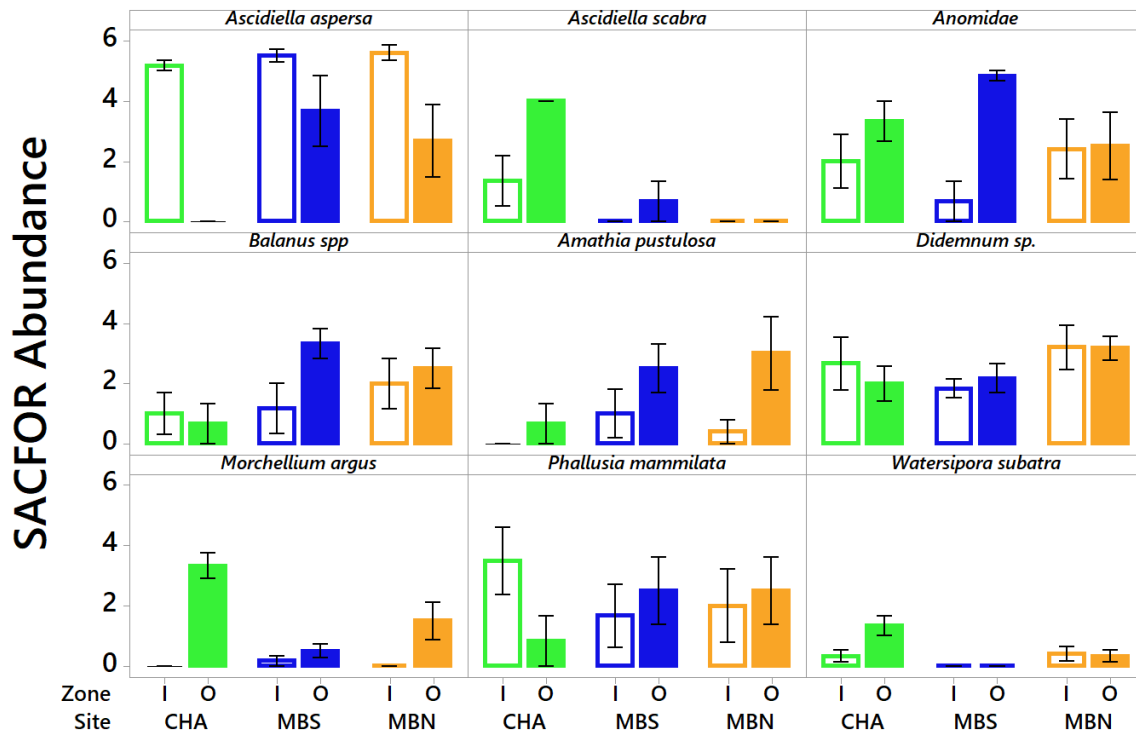


Figure 4: Mean (\pm SE) SACFOR abundances of key species (based on SIMPER analysis) at inner (I: open bars) and outer (O: closed bars) zones of three different marinas within Brest (Château, CHA; Moulin Blanc North, MBN; Moulin Blanc South, MBS). N = 5–6 for each zone

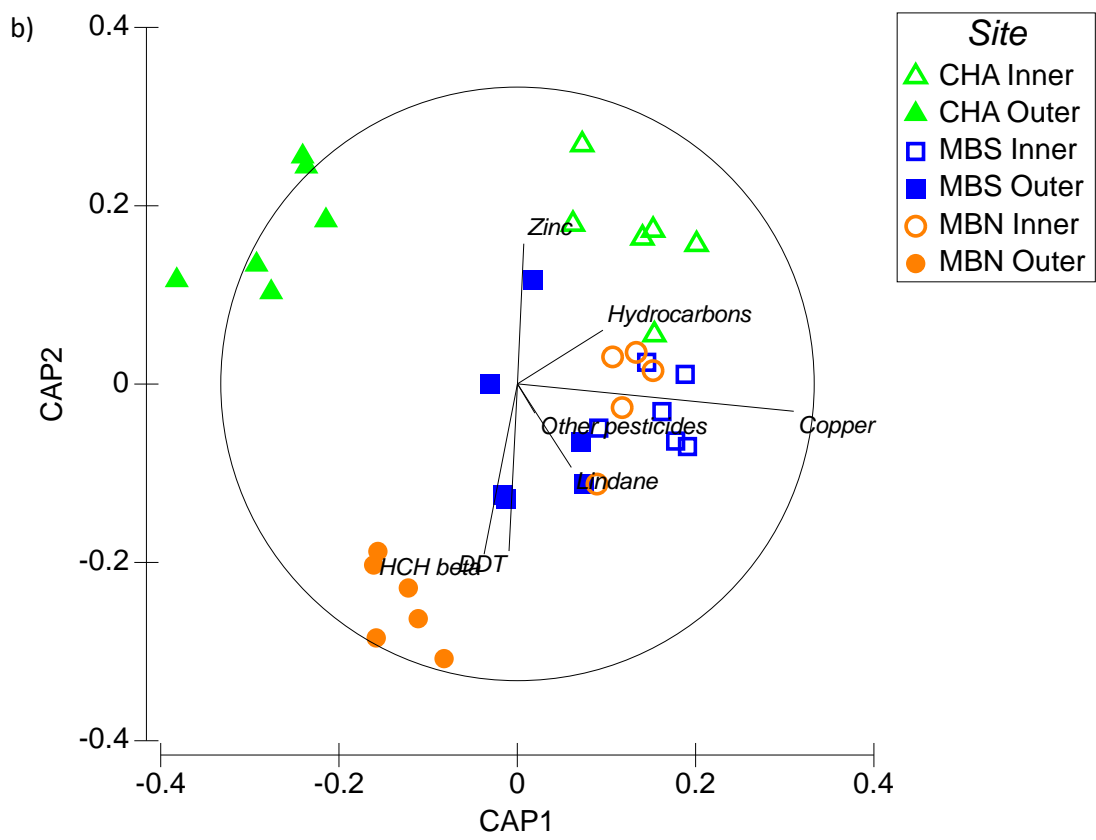
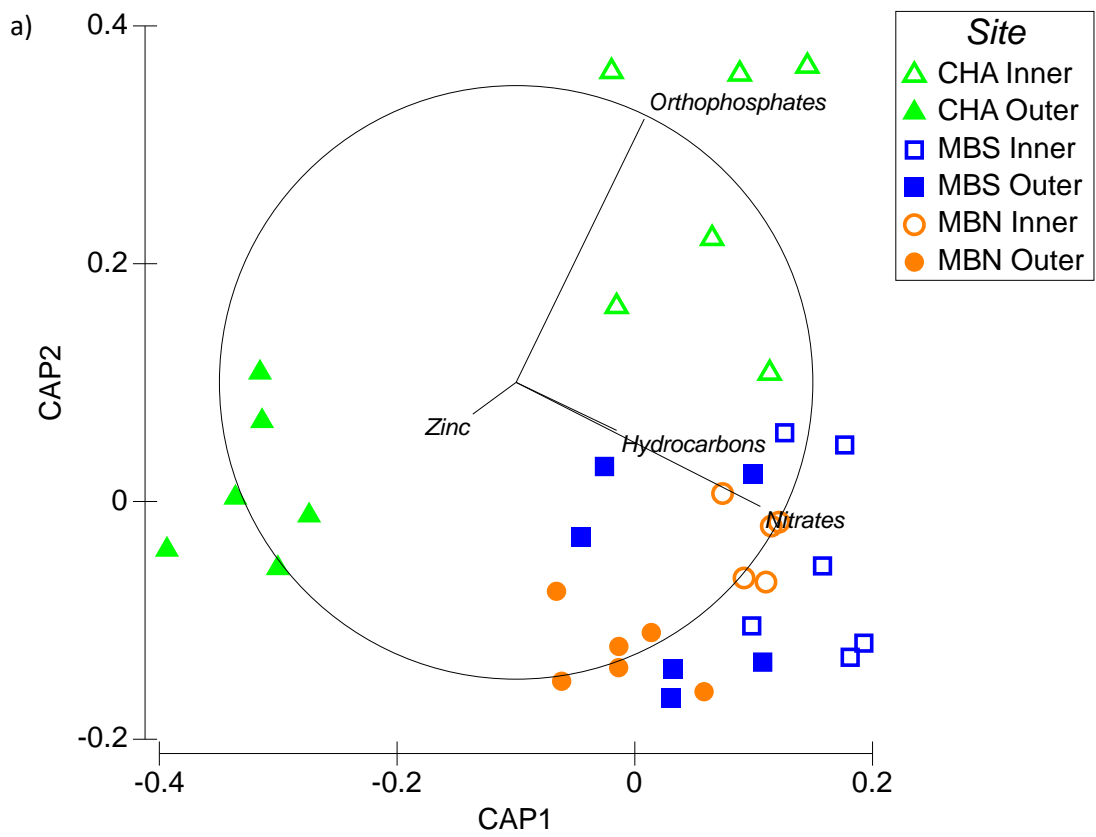


Figure 5: Canonical analysis of principal coordinates (CAP) plot discriminating macrofaunal communities according to site (Chateau – CHA; Moulin Blanc South – MBS; Moulin Blanc North – MBN) and zone (Inner – INT; Outer – EXT), displaying correlations with pollutants collected within the water column (a; $\delta^2 = 0.92$, $p = 0.001$) and within sediments (b; $\delta^2 = 0.94$, $p = 0.001$).

Supplementary material

Table S1: Primer and PCR protocol used for each taxon for DNA barcoding identification

Taxon	Primers (5' -> 3')	PCR conditions
Bryozoa Porifera Asciidiidae Didemnidae Perophoridae Polyclinidae	dgLCO1490 : GGTCACAAATCATAAA ATATTGG [a] dgHCO2198: TAAACTTCAGGGTGA CCAAAAAATCA [a]	95 ° C 5 min + 35 x (95 ° C 50 s + 47 ° C 50 s + 72 ° C 1 min) + 72 ° C 10 min [a]
Styelidae	dgLCO1490 [a] dgHCO2198 [a]	94 ° C 2 min + 35 x (94 ° C 45 sec + 47 ° C 50 s + 72 ° C 55 s) + 72 ° C 5 min [b]
Didemnidae	DidTho2F3: TGCCAAGTTCATCCACATTCTG [c] DidThoR3: TTGCTTTGCTGCTGCCATC [c]	94 ° C 1 min + 35 x (94 ° C 20 s + 56 ° C 30s + 72 ° C 1 min) + 72 ° C 10 min [c]
Didemnidae	Tun F : TCGACTAATCATAAAGATATTA [c] Tun R : AACTTGATTTAAATTACGATC [c]	94 ° C 5 min + 35 x (94 ° C 1 min + 50 ° C 1 min + 72 ° C 1 min) + 72 ° C 7 min [d]
Porifera	Sponge_CO1_F1 : ACATTTTGCTGCCGGTTCAG [e] dgHCO2198: ATAGGDACWGCNTTTA [e]	94 ° C 3 min + 35 x (94 ° C 30s + 40 ° C 30s + 72 ° C 1 min) + 72 ° C 5 min [e]
Porifera	C1Npor2760: TCTAGGTAATCCA GCTAAACC C1J2165: GAAGTTTATATTTAATTTTACC (AGT) GG	92 ° C 2 min + 30 x (92 ° C 30 s + 50 ° C 30 sec 72 ° C 30 s) + 72 ° C 5 min [f]

[a]: Meyer et al. 2005; [b]: Pérez-portela et al. 2009; [c]: Stefaniak et al. 2009; [d]: Ordonez et al. 2015; [e]: Vargas et al. 2015; [f]: Erpenbeck et al. 2002.

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Table S2: Contaminants tested for within the water column of the outer and inner regions of 3 marinas in Brest, France (Chateau: CHA; Moulin Blanc North: MBN; Moulin Blanc South: MBS). < indicates that contaminant was below the detection limit

	Units	Detection limit	CHA Inner	CHA Outer	MBS Inner	MBS outer	MBN Inner	MBN Outer
Nitrates (NO3)	µmol/l	0.1	9	6.8	18	18	24	22
	mg/l	0.0062	0.5561	0.4235	1.101	1.111	1.463	1.371
Orthophosphates (PO4)	µmol/l	0.1	6.1	0.84	0.44	0.35	0.57	0.64
	mg/l	0.0095	0.5793	0.0798	0.0418	0.0332	0.0541	0.0608
Hydrocarbons (C10-C40)	µg/l	100	19000	5100	19000	40000	36000	30000
Zinc	µg/l	1	3.7	1.8	1.8	1.9	6.9	7.6
Copper	µg/l	1	<	<	<	<	<	<
Chlordane-gamma	µg/l	0.005	<	<	<	<	<	<
2,4 -DDE	µg/l	0.005	<	<	<	<	<	<
4,4 -DDT	µg/l	0.005	<	<	<	<	<	<
Dieldrine	µg/l	0.005	<	<	<	<	<	<
HCH Alpha	µg/l	0.005	<	<	<	<	<	<
HCH Beta	µg/l	0.005	<	<	<	<	<	<
HCH-gamma (Lindane)	µg/l	0.005	<	<	<	<	<	<
Endosulfan alpha	µg/l	0.005	<	<	<	<	<	<
Beta-endosulfan	µg/l	0.005	<	<	<	<	<	<
Chlordane-alpha	µg/l	0.005	<	<	<	<	<	<
4,4'-DDD	µg/l	0.005	<	<	<	<	<	<
2,4'-DDD	µg/l	0.005	<	<	<	<	<	<
4,4'-DDE	µg/l	0.005	<	<	<	<	<	<
2,4'-DDT	µg/l	0.005	<	<	<	<	<	<
Endrin	µg/l	0.005	<	<	<	<	<	<
HCH Delta	µg/l	0.005	<	<	<	<	<	<
Heptachlore endo epoxide (Trans)	µg/l	0.005	<	<	<	<	<	<
Heptachlore	µg/l	0.005	<	<	<	<	<	<
Hexachlorobenzene	µg/l	0.005	<	<	<	<	<	<
Aldrin	µg/l	0.005	<	<	<	<	<	<

Table S3: Contaminants tested for within the sediments of the outer and inner regions of 3 marinas in Brest, France (Chateau: CHA; Moulin Blanc North: MBN; Moulin Blanc South: MBS). < indicates that contaminant was below the detection limit

		Units	Detection limit	CHA Inner	CHA Outer	MBS Inner	MBS Outer	MBN Inner	MBN Outer
Nitrate (NO3)	Nitrate (NO3)	mg/kg	20	<	<	<	<	<	<
Orthophosphates (PO4-P)	Orthophosphates (PO4-P)	mg/kg	20	<	<	<	<	<	<
Copper	Copper	mg/kg	5	93.3	44.2	92.6	58.5	83.7	41.5
Zinc	Zinc	mg/kg	5	446	175	162	112	140	108
Hydrocarbons	Total hydrocarbons (C10-C40)	mg/kg	15	1320	156	261	174	341	238
	Hydrocarbons (C10-C16)	mg/kg		52.7	7.2	16.9	6.7	36.9	34.1
	Hydrocarbons (C17-C22)	mg/kg		276	19.1	38.7	31	59.6	41.5
	Hydrocarbons (C23-C30)	mg/kg		529	50.2	89	80	135	71
	Hydrocarbons (C31-C40)	mg/kg		457	80	116	55.8	109	91.8
Organophosphate pesticides	Dichlorvos	mg/kg	0.05	<	<	<	<	<	<
	Diazinon	mg/kg	0.05	<	<	<	<	<	<
	Chlorpyrifos-methyl	mg/kg	0.05	<	<	<	<	<	<
	Methyl Parathion	mg/kg	0.05	<	<	<	<	<	<
	Fenitrothion	mg/kg	0.05	<	<	<	<	<	<
	Malathion	mg/kg	0.05	<	<	<	<	<	<
	Chlorpyrifos-ethyl	mg/kg	0.05	<	<	<	<	<	<
	Ethyl parathion	mg/kg	0.05	<	<	<	<	<	<
	Bromophos-methyl	mg/kg	0.05	<	<	<	<	<	<
	Bromophos-ethyl	mg/kg	0.05	<	<	<	<	<	<
	Ethion	mg/kg	0.05	<	<	<	<	<	<
Organochloride pesticides	HCH Alpha	mg/kg	0.01	<	<	<	<	<	<
	HCH Béta	mg/kg	0.01	<	<	<	<	<	0.05
	HCH, gamma - Lindane	mg/kg	0.01	<	<	0.02	0.17	0.02	0.03
	Hexachlorobenzene (HCB)	mg/kg	0.01	<	<	<	<	<	<
	Heptachlor	mg/kg	0.01	<	<	<	<	<	<
	Aldrin	mg/kg	0.01	<	<	<	<	<	<
	Heptachlor epoxide	mg/kg	0.01	<	<	<	<	<	<
	Endosulfan alpha	mg/kg	0.01	<	<	<	<	<	<
	4,4'-DDE	mg/kg	0.01	<	<	<	<	<	<
	Dieldrin	mg/kg	0.01	<	<	<	<	<	<
	Endrin	mg/kg	0.01	<	<	<	<	<	<
	Beta-endosulfan	mg/kg	0.01	<	<	<	<	<	<
	4,4'-DDD	mg/kg	0.01	<	<	<	<	<	0.01

	2,4'-DDT	mg/kg	0.01	<	<	<	<	<	0.03
	4,4'-DDT	mg/kg	0.01	<	<	0.01	<	<	0.04
	Methoxychlor	mg/kg	0.01	<	<	<	<	<	<
	Isodrine	mg/kg	0.05	<	<	<	<	<	<
	Endosulfan sulfate	mg/kg	0.05	<	<	<	<	<	<
	HCH Delta	mg/kg	0.05	<	<	<	<	<	<
	Chlordane-alpha (cis)	mg/kg	0.01	<	<	<	<	<	<
	Chlordane-gamma (=beta=trans)	mg/kg	0.05	<	<	<	<	<	<
	2,4'-DDD	mg/kg	0.05	<	<	<	<	<	<
	Alachlor	mg/kg	0.01	<	<	<	<	<	<
	Trifluralin	mg/kg	0.05	<	<	<	<	<	<
	2,4' -DDE	mg/kg	0.05	<	<	<	<	<	<
	HCH Epsilon	mg/kg	0.01	<	<	<	<	<	<
Triazine pesticides	Ametryn	mg/kg	0.05	<	<	<	25.1	<	<
	Atrazine	mg/kg	0.05	<	<	<	95.3	<	<
	Atrazine-2-hydroxy	mg/kg	0.05	<	<	<	<	<	<
	Atrazine-Desethyl	mg/kg	0.05	<	<	<	<	<	<
	Atrazine deisopropyl	mg/kg	0.05	<	<	<	<	<	<
	Cyanazine	mg/kg	0.05	<	<	<	<	<	<
	Desethyl-terbutylazine	mg/kg	0.05	<	<	<	<	<	<
	Desmetryn	mg/kg	0.05	<	<	<	<	<	<
	Metribuzin	mg/kg	0.05	<	<	<	<	<	<
	Prometone	mg/kg	0.05	<	<	<	<	<	<
	Prometryn	mg/kg	0.05	<	<	<	2.02	<	<
	Propazine	mg/kg	0.05	<	<	<	9.39	<	<
	Simazine	mg/kg	0.05	<	<	<	25.7	<	<
	Terbumeton-desethyl	mg/kg	0.05	<	<	<	<	<	<
	Terbutylazine	mg/kg	0.05	<	<	<	<	<	<
	Terbutryn	mg/kg	0.05	<	<	<	<	<	<
Urea pesticides	Buturon	mg/kg	0.05	<	<	<	<	<	<
	Chlorbromuron	mg/kg	0.05	<	<	<	<	<	<
	Chlortoluron	mg/kg	0.05	<	<	<	<	<	<
	Desmethyl-isoproturon	mg/kg	0.05	<	<	<	<	<	<
	Diuron	mg/kg	0.05	<	<	<	<	<	<
	Ethidimuron	mg/kg	0.05	<	<	<	<	<	<
	Fenuron	mg/kg	0.05	<	<	<	<	<	<
	Foramsulfuron	mg/kg	0.05	<	<	<	<	<	<
	Iodosulfuron methyl	mg/kg	0.05	<	<	<	<	<	<

	Isoproturon	mg/kg	0.05	<	<	<	<	<	<
	Linuron	mg/kg	0.05	<	<	<	<	<	<
	Methabenzthiazuron	mg/kg	0.05	<	<	<	<	<	<
	Neburon	mg/kg	0.05	<	<	<	<	<	<
	Tebuthiuron	mg/kg	0.05	<	<	<	<	<	<
	Terbumeton	mg/kg	0.05	<	<	<	<	<	<
	Thiazafuron	mg/kg	0.05	<	<	<	<	<	<
	Triasulfuron	mg/kg	0.05	<	<	<	<	<	<