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## Plastic in the inferno: Microplastic contamination in deep-sea cephalopods (*Vampyroteuthis infernalis* and *Abralia veranyi*) from the southwestern Atlantic

Guilherme V. B Ferreira<sup>1\*</sup>, Anne K. S. Justino<sup>1,2</sup>, Leandro Nolé Eduardo<sup>1,3</sup>, Véronique Lenoble<sup>2</sup>, Vincent Fauvelle<sup>2</sup>, Natascha Schmidt<sup>2</sup>, Teodoro Vaske Junior<sup>4</sup>, Thierry Frédou<sup>1</sup>, Flávia Lucena-Frédou<sup>1</sup>.

<sup>1</sup> Departamento de Pesca e Aquicultura da Universidade Federal Rural de Pernambuco – DEPAQ/UFRPE, Rua Dom Manuel de Medeiros, s/n, 52171-900, Recife, Brazil.

<sup>2</sup> Université de Toulon, Aix Marseille Univ., CNRS/INSU, IRD, MIO UM 110, Mediterranean Institute of Oceanography, CS 60584, 83041 – TOULON, France.

<sup>3</sup> Institut de Recherche pour le Développement (IRD), MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Sète, France.

<sup>4</sup> Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP) – Campus do Litoral Paulista. UNESP CLP, Instituto de Biociências, 11380-972 - São Vicente, SP – Brazil.

\* Corresponding author: guilherme.vbf@gmail.com

### HIGHLIGHTS

The first evidence of microplastic in deep-sea cephalopods from the south Atlantic  
The vampire squid is more vulnerable to contamination due to feeding on marine snow  
Depth of foraging influences the size of ingested microplastics  
Most particles extracted from both species were smaller than 1mm  
The migratory behaviour may influence the vertical distribution of microplastics

### ABSTRACT

Microplastics are a relevant environmental concern in marine ecosystems due to their ubiquity. However, knowledge on their dispersion patterns within the ocean basin and the interaction with biota are scarce and mostly limited to surface waters. This study investigated microplastic contamination in two species of deep-sea cephalopods from the southwestern Atlantic with different ecological behaviour: the vampire squid (*Vampyroteuthis infernalis*) and the midwater squid (*Abralia veranyi*). Microplastic particles contaminated most of the evaluated specimens. *V. infernalis* showed higher levels of contamination ( $9.58 \pm 8.25$  particles individual<sup>-1</sup>;  $p < 0.05$ ) than *A. veranyi* ( $2.37 \pm 2.13$  part. ind.<sup>-1</sup>), likely due to the feeding strategy of *V. infernalis* as a particulate organic matter feeder. The size of extracted microplastics was inversely proportional to the depth of foraging. The microplastics were highly heterogeneous in composition (shape, colour and polymer type). Our results provide information regarding microplastic interaction with deep-sea organisms and evidence of the biological influence in the microplastic sinking mechanism.

Keywords: Plastics, Mesopelagic zone, Marine Litter, Deep-sea squids, Oceanic islands.

Plastics have a crucial role in the global economy as raw materials, positively influencing the market dynamics due to the low production costs, high durability, and versatility (Andrady and Neal, 2009; Derraik, 2002). However, during the last decades, the great demand and further production of plastics have rapidly increased (Geyer et al., 2017), arising major concerns regarding their final fate and hazardous potential. Microplastics (<5 mm; MPs) (Arthur et al., 2009) have gained attention due to their toxicity and the widespread occurrence as a contaminant in the aquatic environment (Bhagat et al., 2020; Eriksen et al., 2014).

Inshore economic activities yield the major waste production. Continental areas are the main plastic reservoir in the biosphere (GESAMP, 2016; Van Sebille et al., 2015), where a considerable portion of these products are mismanaged along the production chain or disposed into landfills, then later flushed by rainfall into waterways (Jensen et al., 2019). Improper sewage disposal, inefficient wastewater treatment plants, and atmospheric transport are additional sources of MPs to the aquatic ecosystems (Allen et al., 2019; De Falco et al., 2019). Once in the drainage basin, advection flow will transport MPs downstream to the estuary (Lima et al., 2014). Estuaries can supply many MPs to the adjacent coastal waters and are acknowledged as the main source to the ocean (Lebreton et al., 2017), mostly during increased river flow periods (Cheung et al., 2016).

Microplastics are ubiquitous at the ocean surface (Hardesty et al., 2017), drifting according to the wind, geostrophic currents, Stokes drift, and surface currents that will lead to convergence areas (Jiang et al., 2020; Mountford and Morales Maqueda, 2019; Van Sebille et al., 2015). The so-called hot spots of MP pollution tend to be correlated with ocean gyres and polar zones (Jiang et al., 2020; Lima et al., 2021). Peaks in the density (particles/m<sup>3</sup>) of this contaminant are also associated with the proximity of the main sources, mostly enclosed seas surrounded by densely inhabited areas (Van Sebille et al., 2015). However, several aspects related to the occurrence, density and dispersion of MPs into deeper ocean layers are still poorly understood.

The density of MPs in the deep sea relies on thermohaline currents (Kane et al., 2020) and a complex sinking pattern, which encompasses oceanographic parameters, specific features of particles (*e.g.*, the density of polymers, surface area, and fragmentation rates), and incorporation by marine snow and faecal pellets (Onink et al., 2019; Kvale et al., 2020). Microplastics comprise a heterogeneous assemblage of particles that vary in shape, colour, size and chemical composition, including several

polymer derivatives of the petrochemical industry (Barnes et al., 2009). Most polymers have a molecular density higher than seawater and are thus negatively buoyant (Mountford and Morales Maqueda, 2019). Advection flow might play a crucial role in their sinking rates (Kvale et al., 2020). However, some polymers (e.g., polyethylene, polystyrene, and polypropylene) (Geyer et al., 2017) are positively buoyant and likely require additional mechanisms and/or greater residence time to finally sink through the water column (Koelmans et al., 2017; Long et al., 2015).

Due to their prevalence, persistence, diminutive size, and colonisation by microorganisms, MPs are highly susceptible to be ingested throughout the food web (Everaert et al., 2018; Ferreira et al., 2019b; Roch et al., 2020). Moreover, marine organisms play an important role in vertical MP transportation (Kvale et al., 2020). Zooplankton modifies sinking rates of egested MPs through their incorporation within faecal pellets (Cole et al., 2016). Vertical migratory species from the mesopelagic realm induce important vertical biomass fluxes (Davison et al., 2013; Eduardo et al., 2020). Thereby, the diel migration pattern of preying upon contaminated resources (contaminated habitat and food) in the uppermost ocean layers and returning to the deep layers suggests that vertical migration contributes to MP transportation.

Despite providing key ecological links between different depth strata (Robinson et al., 2010), cephalopods are among the least studied groups regarding MP contamination (Alejo-Plata et al., 2019; Oliveira et al., 2020). Yet, surveys have pointed to microplastic contamination among the most common preys (copepods, euphausiids, crabs, tunicates, and fishes) and predators (cetaceans) of deep-sea cephalopods (Choy et al., 2019; Davison and Asch, 2011; Desforges et al., 2015; Lusher et al., 2015; Wieczorek et al., 2018). Cephalopods are exposed to at least two main contamination pathways; the contaminants present in the water column and those in the prey's digestive tract. *Vampyroteuthis infernalis* (Chun, 1903) is the only living species from the Vampyroteuthidae family. It usually inhabits meso- and bathypelagic zones throughout the oceans (Hoving and Robison, 2012) with a slow-swimming and opportunistic behaviour foraging on copepods, but mostly on marine snow (Golikov et al., 2019). The Eupoloteuthidae *Abralia veranyi* (Rüppel, 1844) inhabits the upper layers of the mesopelagic zone in the Atlantic Ocean, migrating towards the epipelagic zone to prey on copepods, decapods, and fish (Guerra-Marrero et al., 2020).

The objectives of this study were to (i) evaluate if cephalopods from the southwestern tropical Atlantic (*V. infernalis* and *A. veranyi*) are contaminated by

microplastic particles and (ii) investigate whether cephalopods with different ecological behaviour exhibit different patterns of contamination. To our knowledge, this is one of the first studies to evaluate microplastic contamination in deep-sea cephalopods from the South Atlantic and the first to evaluate contamination in *V. infernalis* and *A. veranyi*.

Mesopelagic cephalopods were collected in the southwestern tropical Atlantic during the multidisciplinary research cruise ABRACOS2 (Acoustics along the BRAZilian COaSt 2), onboard the French R/V Antea from 9<sup>th</sup> April to 6<sup>th</sup> May 2017 (Bertrand, 2017) (Fig. 1). Samplings were carried out off northeastern Brazil using a micronekton trawl net with different mesh sizes (body mesh: 40 mm, cod-end mesh: 10 mm, estimated opening area: 120 m<sup>2</sup>). Individuals were selected from 5 sampling stations, and vertical trawls were conducted both day and night, between 25 and 1,113 m depth at 2-3 kt (Eduardo et al., 2020).

The study area encompasses the Fernando de Noronha Archipelago, Rocas Atoll, and adjacent seamounts (Fig. 1), which together are considered an Ecologically and Biologically Significant Marine Area and include Marine Protected Areas (CBD, 2014). Additionally, Fernando de Noronha Archipelago is classified as an UNESCO Natural Heritage of Humanity, and the number of tourists reaches >50,000 in the peak season (austral summer season) (IBGE, 2010). The surface waters of the study area are not estimated to be an accumulation zone for MPs (Jiang et al., 2020; Lima et al., 2021); the area is dominated by the South Equatorial Current (SEC) and South Equatorial Undercurrent (SEUC) and is characterised by a thin thermocline and high stratification (Assunção et al., 2020).

Promptly upon collection at each sampling station, specimens (*V. infernalis* n=19 and *A. veranyi* n=8) were fixed in a 4% formalin solution and then preserved in a 70% ethanol solution in the laboratory. Organisms were identified (Nesis, 1982; Roper et al., 1984), measured (nearest 0.001 cm of total length and dorsal mantle length) and weighted (nearest 0.0001 g of total weight). Since precautionary measures to avoid contamination were not implemented during sampling and storage, individuals were thoroughly washed before analysis with filtered (glass fibre filter) distilled water to remove any particles attached to the outermost tissue. Then, beaks were removed, and the entire animal was submitted to chemical digestion through sodium hydroxide (NaOH 1 mol/L; PA 97%).

We implemented chemical digestion through base reaction (NaOH), due to greater logistical performance (short incubation period and cost-effective method) and for preserving the integrity of polymers after chemical digestion (Budimir et al., 2018). Specimens were individually stored in beakers covered by glass lids. Each beaker was filled with NaOH solution (100 mL of solution per gram of organic sample) and oven-dried at 60°C for 24 h (Lusher et al., 2017; Wieczorek et al., 2018). Samples were mixed with a glass stick two times during the incubation period to homogenise the solution.

Samples (output of digestion process) were filtered through a glass fibre filter (GF/F 0.7 µm pore size Whatman) using a membrane filtration system with a vacuum set up (equipped with laboratory glassware). The glass fibre filters with the digestion residues were then placed in covered Petri dishes and oven-dried at 60°C for 24 h (for the complete protocol, see Justino et al., 2021). Two observers visually examined samples to reduce identification bias (overestimation and/or underestimation of contaminants) on a stereomicroscope (Zeiss Stemi 508, using 40-50x magnification with a detection limit of 20 µm) coupled with a device camera (Axiocam 105 Color). Items suspected to be microplastics were photographed, counted, measured (Zeiss Zen 3.2), and categorised according to morphological and optical characteristics into (i) fibres (filamentous shape), fragments (thick with an irregular shape), film (flat with an irregular shape), foam (soft with an irregular shape) and beads (spherical shape), and (ii) black, blue, green, red and white (Lusher et al., 2017). Petri dishes were kept closed during the entire identification process to avoid airborne contamination of samples.

As chemical digestion is not an efficient process to distinguish between plastic fibres and cellulose fibres, a sub-sample of particles extracted from the specimens were identified through Laser Directed Infra-Red analysis (LDIR). The selection of particles composing the sub-sample followed their shape as criteria rather than differences between the species. The absorbance of polymers was obtained using the Agilent 8700 LDIR Chemical Imaging System with the Microplastics Starter 1.0 library. Each spectral curve resulted from scans performed in the wavelength ranging from 1800 to 975 cm<sup>-1</sup> (Ourgaud et al., *In prep.*). The specific polymer was asserted when a given particle registered above 70% of similarity with the reference spectrum.

Several procedures were taken to avoid airborne contamination of samples. All analyses were conducted under a laminar flow cabinet in a subsection of the laboratory designed to limit the flow of people (Wieczorek et al., 2018). As clothing made from

synthetic fibres represents a potential source of contamination (Su et al., 2019), latex gloves, 100% cotton lab coats, and facemasks were worn during laboratory analysis.

All solutions used during the MP extraction were made using distilled water and filtered over a 47 mm GF/F filter (0.7  $\mu\text{m}$  pore size). Before the analysis, work surfaces, equipment, and manipulation instruments were thoroughly cleaned with 70% alcohol. All manipulation instruments were rinsed with filtered distilled water and double-checked under a stereomicroscope for contamination before use.

A procedural blank was run in conjunction with each sample batch (~5 samples) to determine airborne contamination during the analysis. For blanks, a beaker was filled with 300 ml of NaOH solution and submitted to the same procedures applied to the samples (Justino et al., 2021). Out of the five procedural blanks implemented during the analysis, two registered contamination, a single blue fibre. Any contamination evinced from the procedural blanks was subtracted from the corresponding sample batch (1 blank for each ~5 samples). Thereby, particles matching these characteristics (shape and colour) were omitted from the dataset and analysis.

The Percentage of the Frequency of Occurrence (FO%) was calculated to assess the overall contamination status of the species. The FO% was calculated as the percentage of individuals in a given species in which MP particles were recorded. As the data on MPs extracted did not meet parametric assumptions, Mann-Whitney tests were applied to determine whether there were any differences in the number and size (shape, colour and the overall particles, regardless of shape and colour) of MPs between the species. The number of extracted MPs was expressed as abundance (particles individual<sup>-1</sup>), containing all analysed individuals, including those that did not ingest MPs (Provencher et al., 2017). All analyses were carried out using R 3.6 (R Core Team, 2020) with a 5% significance level.

In this study, two species of deep-sea cephalopods were analysed. Both species registered a high prevalence (FO%) of MPs (*V. infernalis* 100% and *A. veranyi* 87.5%) (Table 1). Contamination levels (number of particles) varied between the species, ranging from 1 to 33 particles for *V. infernalis* (a total of 182 particles) and from 0 to 7 particles for *A. veranyi* (a total of 19 particles). *Vampyroteuthis infernalis* ( $9.58 \pm 8.25$  part. ind.<sup>-1</sup>; Mean  $\pm$  Standard deviation) ( $W = 134.5$ ,  $p \leq 0.05$ ) was the more contaminated species (*A. veranyi*  $2.37 \pm 2.13$  part. ind.<sup>-1</sup>) (Fig. 2 and Supplementary material).

Plastic particles extracted from cephalopods ranged from 0.06 to 3.91 mm in size. Most particles were categorised within the MP size range (99% of particles < 5 mm). Independent of shape and colour, the average length of particles extracted from *A. veranyi* ( $0.63 \pm 1.19$  mm) ( $W = 116, p \leq 0.05$ ) was longer than that of *V. infernalis* ( $0.61 \pm 0.34$  mm) (Fig. 2 and Supplementary material).

In total, 201 MP particles corresponding to five main groups were recorded (Fig. 3). Fragments were the most common shape of MP extracted for both species (*V. infernalis*  $3.36 \pm 3.67$  part. Ind.<sup>-1</sup> and *A. veranyi*  $1.5 \pm 1.69$  part. ind.<sup>-1</sup>), followed by fibres [ $3.05 \pm 2.34$  part. Ind.<sup>-1</sup> ( $W = 120.5, p \leq 0.05$ ) and  $0.5 \pm 0.75$  part. ind.<sup>-1</sup>, respectively] and beads ( $1.84 \pm 6.6$  part. Ind.<sup>-1</sup> and  $0.37 \pm 0.52$  part. ind.<sup>-1</sup>, respectively) (Table 1). Microplastics categorised as foam ( $1.21 \pm 2.01$  part. ind.<sup>-1</sup>) and films ( $0.1 \pm 0.31$  part. ind.<sup>-1</sup>) were only recorded in *V. infernalis* (Table 1 and Supplementary material).

The MP shapes did not significantly differ in size according to species. Among the different shapes, fibres showed the broadest size range for both species (*V. infernalis*  $0.91 \pm 0.92$  mm and *A. veranyi*  $0.64 \pm 1.23$  mm) (Fig. 2). Fragments (*V. infernalis*  $0.33 \pm 0.37$  mm and *A. veranyi*  $0.13 \pm 0.14$  mm) and foam (*V. infernalis*  $0.16 \pm 0.22$  mm) were mostly ingested as small particles, whereas film (*V. infernalis*  $0.01 \pm 0.04$  mm) and beads (*V. infernalis*  $0.04 \pm 0.06$  mm and *A. veranyi*  $0.03 \pm 0.04$  mm) were the smallest fractions of ingested MPs (Fig. 2 and Supplementary material).

Concerning the colour of extracted MPs, white particles ( $6 \pm 7.17$  part. ind.<sup>-1</sup>) prevailed in *V. infernalis* ( $W = 84.5, p \leq 0.05$ ), followed by blue ( $1.26 \pm 2.15$  part. ind.<sup>-1</sup>) and red ( $1.15 \pm 1.64$  part. ind.<sup>-1</sup>), whereas *A. veranyi* was most contaminated by blue ( $1.12 \pm 1.35$  part. ind.<sup>-1</sup>), white ( $0.87 \pm 0.64$  part. ind.<sup>-1</sup>) and black particles ( $0.25 \pm 0.7$  part. ind.<sup>-1</sup>) (Table 1 and Supplementary material).

The subsample of particles analysed by LDIR comprehended 5.5% of the total particles extracted from the cephalopods. LDIR analysis successfully identified 78% of the subset as plastic polymers, including seven different polymers (polyethylene, polyethylene terephthalate, polyvinyl chloride, polyamide, styrene-butadiene rubber, chlorinated polyisoprene and polyurethane) (Fig. 4). Cellulose and wool particles were not registered in the subsample tested, but 22% of particles could not be identified through the assessed library. Thereby, those particles can be marine debris of natural origin or highly weathered plastic polymers.

Plastic pollution has become a major environmental issue (Eriksen et al., 2014; Raubenheimer and McIlgorm, 2018). Microplastics are asserted to contaminate the soil, air, and water bodies, which are the main pathway for their dispersion and accumulation (Allen et al., 2019; Corradini et al., 2019; Lebreton et al., 2017). Despite the recent awareness of the ubiquity of MPs, little is known regarding the general patterns of dispersion, fragmentation, and interaction with marine biota in the deepest ocean layers (Kane et al., 2020; Liu et al., 2020; Porter et al., 2018). Indeed, plastics (> 330  $\mu\text{m}$ ) contaminating the upper ocean layers represent less than 1% of the estimated global annual plastic inputs to the ocean (Jambeck et al., 2015; Van Sebille et al., 2015). The remaining missing plastics are likely fragmenting into smaller pieces, diffusing into deeper layers, sediments (Dai et al., 2018; Courtene-Jones et al., 2017; Gerigny et al., 2019; Kane et al., 2020; Kanhai et al., 2018) and being assimilated by deep-sea biota (Choy et al., 2019; Sathish et al., 2020).

Cephalopods inhabiting coastal areas have been asserted to ingest MPs (Daniel et al., 2021; Oliveira et al., 2020). Oliveira et al. (2020) identified higher contamination levels (number of particles) in wild cuttlefish (*Sepia officinalis*) than in those from a cultured marine station on the coast of the Iberian Peninsula, likely due to the retention of particles in the filtration system of culture tanks. Daniel et al. (2021) investigated MPs in the edible tissues of four shellfish species on the Arabian Sea coast and detected the highest levels of contamination in the squids (*Uroteuthis duvaucelii*). This is the first study to investigate MPs in deep-sea cephalopods from the southwestern Atlantic and to provide information on *V. infernalis* and *A. veranyi*. Surveys on the deep-sea are logistically challenging, and literature in this field is scarce. To our knowledge, only two studies, both regarding macroplastics (>5 mm), have been published on deep-sea representants of this group (Braid et al., 2012; Rosas-Luis, 2016). Both studies focused on the jumbo squid *Dosidicus gigas*, describing ingestion of large particles, mainly fishing lines. Braid et al. (2012) also observed the ingestion of plastic beads, but the dimension of particles was not assessed. Thereby, comparisons between our findings and other studies on deep-sea cephalopods are limited.

Microplastic ingestion is correlated with the density (particles  $\text{m}^{-3}$ ) of contaminants in the environment (Güven et al., 2017), which can significantly differ according to depth, turbulence, and specific areas within the ocean basin (Kooi et al., 2017; Lima et al., 2021; Reisser et al., 2015). The feeding strategy of species is also influenced by the levels of contamination (Ferreira et al., 2019a; McNeish et al., 2018;

Messinetti et al., 2018; Miller et al., 2020; Mizraji et al., 2017; Setälä et al., 2014). Marine biota can ingest MPs through three main pathways (Moore, 2008): (i) actively when they are mistaken for actual prey (Moore, 2008), which in this case are very similar in size (copepods, mysids, and early-stage decapods) (Figueiredo et al., 2020) or (ii) passively, through particles adhered and in the surroundings of prey during the feeding process, such as suggested for cephalopods in field studies (Alejo-Plata et al., 2019; Rosas-Luis, 2016). Additionally, MPs can also be (iii) transferred within the trophic web by ingesting contaminated prey (Ferreira et al., 2019b; Nelms et al., 2018; Setälä et al., 2014). Indeed, the trophic transfer has been acknowledged as the core pathway for MP ingestion in cephalopods (Daniel et al., 2021; Braid et al., 2012); this group, in turn, may also act as vectors transferring MPs to higher trophic levels (Alejo-Plata et al., 2019) and potentially to humans as seafood (Daniel et al., 2021).

Both species analysed here (*A. veranyi* and *V. infernalis*) exhibited a high prevalence of MPs (FO% and part. ind<sup>-1</sup>) when compared to other deep-sea biota (Braid et al., 2012; Daniel et al., 2021; Davison and Asch, 2011; Rosas-Luis, 2016; Wieczorek et al., 2018). MP ingestion was observed among marine copepods, euphausiids, crabs, tunicates, and mesopelagic fishes that are common prey for the species analysed in this study (Bernal et al., 2020; Choy et al., 2019; Desforges et al., 2015; Lusher et al., 2016; Sathish et al., 2020; Wieczorek et al., 2018), which indicates the possibility of this contamination pathway.

The species analysed in this study have different ecological behaviour. *Abralia veranyi* inhabits the upper mesopelagic zone (200-500 m depth) during daytime and migrates towards the epipelagic zone to forage during the night-time (Roper et al., 1984) as part of the DSL (Deep Scattering Layer) migrants. According to the size range of captured *A. veranyi*, most of the individuals were under ontogenetic shift, reaching the adult phase (dorsal length > 30.9 mm), when they prey mostly on zooplankton and fish (Guerra-Marrero et al., 2020). In general, MP occurrence is greater in surface and sub-surface waters than in intermediate layers (Bagaev et al., 2018; Kvale et al., 2020; Zobkov et al., 2019). *Abralia veranyi* forage on the ocean layer with the higher density of MPs and feeds on copepods and fishes, both frequently reported to ingest MPs (Bernal et al., 2020; Botterell et al., 2019; Wieczorek et al., 2018); hence the recurrent and relatively high contamination levels observed are not surprising. Contamination levels were higher than those observed for the large predatory squid *D. gigas* (Braid et al., 2012; Rosas-Luis, 2016), which forages in deeper layers, but lower than those for *S.*

*officinalis* that feeds in shallower coastal waters (Oliveira et al., 2020). The feeding habitat (epipelagic zone) might also have influenced the size of MPs ingested by *A. veranyi*. This species showed the longest MPs, and the size of these particles in the water column is negatively correlated with depth (Dai et al., 2018; Zobkov et al., 2019).

*Vampyroteuthis infernalis* inhabits deeper layers from the lower mesopelagic zone (500-1000 m depth) (Hoving and Robison, 2012). Individuals analysed in this study corresponded to the juvenile phase (dorsal length < 66 mm) (Hoving et al., 2015; Schwarz et al., 2020), when they display a transition in the locomotion pattern towards a more passive predatory behaviour, feeding on aggregated marine snow and POM (Particulate Organic Matter) associated feeder invertebrates (Golikov et al., 2019; Hoving and Robison, 2012). To a smaller degree, *V. infernalis* juveniles also actively prey upon zooplankton (Seibel et al., 1998).

Despite MPs being less available in the foraging habitat of *V. infernalis*, this species registered higher contamination levels than *A. veranyi*, other cephalopod species (Braid et al., 2012; Daniel et al., 2021; Rosas-Luis, 2016) and fishes (Boerger et al., 2010; Lusher et al., 2016; Wieczorek et al., 201), which specific ecological traits might explain. Marine aggregates (e.g., marine snow and faecal pellets) play an essential role in transporting MPs from the surface to the deep ocean (Cole et al., 2016; Kvale et al., 2020; Möhlenkamp et al., 2018). Thereby, *V. infernalis*, feeding on faecal pellets and marine snow (Golikov et al., 2019; Hoving and Robison, 2012), is likely more vulnerable to contamination. Additionally, *V. infernalis* is asserted to have the lowest metabolic rates among all cephalopods, as an adaptative trait to live within the oxygen minimum zones (OMZs) (Seibel et al., 1997), contributing to low egestion rates, which may result in a momentary build-up of MP particles in the body.

The MPs extracted from *A. veranyi* and *V. infernalis* were heterogeneous, varying in shape and colour. However, no differences were observed between species, suggesting the absence of active ingestion of particles or, at least, a similar “preference” for the same colours and shapes. Nevertheless, according to the size of the specimens and the tiny dimensions of the particles, active predation (confusion with actual prey) is unlikely to occur. The colour composition of MPs extracted from cephalopods resembled those observed in epipelagic and deep-sea water samples elsewhere (Arctic and Atlantic Ocean) (Campos da Rocha et al., 2021; Courtene-Jones et al., 2017; Kanhai et al., 2018).

Fragments were the most common shape of MP ingested for both species, as observed for *U. duvaucelii* and deep-sea fishes from the North Pacific Central Gyre, Northeast Atlantic and Eastern Mediterranean (Anastasopoulou et al., 2013; Boerger et al., 2010; Daniel et al., 2021; Pereira et al., 2020). The contamination by fibres was very relevant, being the second most common shape extracted and prevailing among the larger particles (>1 mm), likely due to their high density in the environment. Fibres comprehend more than 90% of MPs in the water column (Kanhai et al., 2018; Lima et al., 2021) and generally is the most common shape ingested by deep-sea organisms (Gago et al., 2020; Oliveira et al., 2020; Sathish et al., 2020; Wieczorek et al., 2018). Despite beads being seemingly less common in water samples (Barrows et al., 2018), the spherical MPs were also observed in *D. gigas* (Braid et al., 2012). Beads have the highest sinking rate among the different MP shapes (Khatmullina and Isachenko, 2017), positively influencing their density (particles m<sup>-3</sup>) in the deep sea.

Given the size range of extracted MPs, they are likely not bioaccumulated, since only the smaller fraction of microplastics (< 5 µm) and nanoplastics (< 1 µm) are prone to be assimilated into tissues and organs (Lee et al., 2019; Lu et al., 2016; Sökmen et al., 2020). However, particles within this size fraction could not be assessed using the methodology implemented herein.

The assimilation of plastics is associated with the uptake of several chemical additives (*e.g.*, plasticisers, pigments, flame retardants, and UV stabilisers) introduced during the manufacturing and pollutants adsorbed from the environment (Batel et al., 2016; Galloway et al., 2017). The large surface area and the hydrophobic nature of MPs increase the sorption capability of several organic pollutants (*e.g.*, DDT, PCBs and PAHs) (Bakir et al., 2012; Rios et al., 2007; Rochman et al., 2013), whereas the biofouling of MPs enhances the sorption of heavy metals and antibiotics (Richard et al., 2019; Wang et al., 2020). The sorption of pollutants is influenced by environmental conditions, polymer composition, and residence time; plastics with longer stays in the ocean accumulate higher concentrations of pollutants (Rochman et al., 2014). Polyethylene, for instance, the most common polymer extracted from the cephalopods in this study, is asserted to have a greater sorption capability than other polymers (Alimi et al., 2018).

The role of MPs as vectors for pollutants and their adverse effects on cephalopods has not been evaluated to date. However, experimental research on MP ingestion by fish indicates several adverse effects (*e.g.*, intestinal damage, oxidative stress, immunotoxicity, genotoxicity, developmental, reproductive and locomotor toxicity)

(Bhagat et al., 2020). Further research is necessary to investigate if MP contamination can reduce the swimming and feeding activity of cephalopods, as was observed for fish (de Sá et al., 2015; Qiang and Cheng, 2019). If so, deep-sea cephalopods could be more vulnerable to the adverse effects given the extended diel vertical migration to forage.

Understanding general MP dispersion into the deep ocean and their final fate is one of the most relevant open questions in the field. The broad range of shapes and identified polymers indicate multiple sources of contamination. The biological activity influences the MP sinking rates through biofouling, aggregation in marine snow and faecal pellets. Additionally, vertical migratory species may play a significant role. Cephalopods and other vertical migrants ingest MPs in shallower waters with high MP density and likely egest those contaminants into deeper layers during resting periods.

Cephalopods are widely recognised as playing a crucial role in many marine ecosystems, both as predators and prey. The vampire and midwater squids are important prey for harvestable fish stocks and highly threatened species of the southwestern Atlantic (Vaske et al., 2009, 2012). Yet, they remain poorly known worldwide while increasingly at risk in several ways, such as climatic change (Levin et al., 2019) and deep-sea exploitation (Drazen et al., 2020). Additionally, we show that both *A. veranyi* and *V. infernalis* are highly contaminated by MPs, which threatens their survival and may enhance the plastic transportation between oceanic layers.

With the observed pervasion of plastic into the ocean, we reaffirm that the structure and function of deep-sea ecosystems could undergo changes that, given the current state of knowledge, may go mostly unnoticed by scientists and marine resource managers. More studies encompassing a greater sample size, various sampling depths, oceanographic parameters, and different taxonomic and ecological groups are required to understand the interaction with MPs in these habitats and investigate if the driving force responsible for the biological pump could also “sequester” microplastics into deeper ocean layers, as a plastic pump.

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#### **DECLARATION OF COMPETING INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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## FIGURE CAPTIONS

**Figure 1** – Study area in the southwestern tropical Atlantic, sampling stations where *V. infernalis* ● and *A. veranyi* ■ were captured (5 stations).

**Figure 2** – Mean ( $\pm$ standard error) (a) number, (b) length, and (c) (%) shape according to the size of ingested microplastics by *V. infernalis* and *A. veranyi* from the southwestern tropical Atlantic.

**Figure 3** – Microplastic particles extracted from deep-sea cephalopods (*V. infernalis* and *A. veranyi*): (a) fibre, (b) tangled fibres, (c) fragment, (d) foam, (e) film and (f - g) beads.

**Figure 4** – Microplastic polymers identified by the LDIR analysis: (a) particle composition, (b) types of plastic polymers, (c) PVC (polyvinyl chloride) MP fragment (solid line: particle spectrum; dotted line: reference spectrum), (d) PET (polyethylene terephthalate) MP fibre (solid line: particle spectrum; dotted line: reference spectrum), (e) PE (polyethylene) MP foam and (f) PU (polyurethane) MP fragment.