Antifouling paint particles in intertidal estuarine sediments from southwest England and their ingestion by the harbour ragworm, *Hediste diversicolor*

by

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Abstract

Antifouling paint particles (APPs) of between 500 μm and > 2 mm in diameter have been identified in silty, intertidal estuarine sediments through a combination of microscopy and x-ray fluorescence spectrometry. APPs were heterogeneously distributed, with maximal concentrations of 430 particles L^{-1} (0.2 g L^{-1}) near to a facility where boats are regularly maintained and 400 particles L^{-1} (4.2 g L^{-1}) at a location where old boats had been abandoned, with the majority of particles encountered in the finest size fraction retrieved. APPs contained variable concentrations of Cu, Zn, Sn and Pb, with respective maxima of 562,000, 269,000, 9,970 and 126,000 mg kg^{-1}. These characteristics are attributed to a multitude of contemporary and historic sources of an assortment of formulations and result in significant but heterogeneous metal contamination of local sediments. APPs were also identified in the guts of the deposit-feeding ragworm, *Hediste diversicolor*, that inhabited sediments impacted by abandoned boats or boating activities. The tissue of *H. diversicolor* was particularly enriched in Cu where ingested APPs were observed, with a significant correlation between Cu concentrations in the two media (r = 0.734) presumably reflecting the inability of the animal to regulate this metal. While the toxicity of APPs requires further investigation, there is clearly a need for stricter regulations on antifouling wastes in boatyards and marinas and a requirement to better safeguard abandoned boats.

Keywords: antifouling paint particles; microplastic; estuarine sediment; copper; zinc; benthic organisms

Capsule: Antifouling paint particles in contaminated estuarine sediments can be ingested by *Hediste diversicolor* and result in the bioaccumulation of copper

1. Introduction

Marine biofouling is caused by the accumulation of unwanted organisms on submerged structures. Adverse impacts of biofouling include additionalcleaning and maintenance costs and increased frictional drag on boats, leading to higher fuel consumption and reduced manoeuvrability (Chambers et al., 2006). Antifouling paint is applied to many marine structures to reduce biofouling and typically works by leaching or eroding biocides into the surrounding environment (Yebra et al., 2004). Organotin-based formulations were popular antifoulants for several decades until significant, adverse effects on non-target species were demonstrated (Bailey and Davies, 1991; Axiak et al., 2000). The subsequent phasing out of antifouling paints containing organotins resulted in the development of new, tin-free paints, with most contemporary antifouling paints containing Cu(I) as the main biocide, often in combination with Zn-based compounds such as zinc oxide (ZnO), and a combination of booster biocides that include zinc pyrithione (ZnPT), Irgarol 1051 and diuron (Chambers et al., 2006).

Although the environmental impacts and toxicity of antifouling paints applied to boats and marine structures have been studied extensively (Comber et al., 2002; Karlsson et al., 2010; Bao et al., 2013; Gallo and Tosti, 2015), the effects of spent antifouling paint particles (APPs) in the marine environment are less well known. Significant quantities of APPs are generated in boatyards and marinas during maintenance and cleaning of boat hulls. In the UK and many other countries the disposal of APPs is largely unregulated in the recreational (but not commercial) boating sector, and as such APPs are often transported from hard-standings and slipways into the local marine environment (Turner, 2010). APPs also originate from weathering of abandoned or beached boats, which are often coated in numerous layers of historic antifouling formulations (Rees et al., 2014); significantly, these formulations may include biocides that have been banned or that are no longer used, including organotin-based chemicals as well as compounds of arsenic, lead and mercury (Turner and Rees, 2015). Once in the marine environment, APPs can accumulate in benthic sediments around marinas, boatyards and abandoned boats and act as a secondary, localised and heterogeneous source of biocides both in particulate and aqueous form (Thomas et al., 2003; Turner et al., 2008a; Soroldoni et al., 2018a).

Benthic organisms are essential for the functioning of marine coastal ecosystems and play an important role in energy transfer between pelagic and benthic ecosystems. Controlled laboratory studies have shown that exposure to APPs can lead to the accumulation of the biocidal metals, Cu and Zn, in the tissues of the common mussel *Mytilus edulis* (Turner et al., 2009), the common periwinkle *Littorina littorea* (Gammon et al., 2009) and the lugworm *Arenicola marina* (Turner et al., 2008b). Uptake of metals appears to take place through both aqueous exposure to APP leachate or via direct ingestion of APPs, with organisms unable to differentiate APPs from their food. What has been lacking, however, is evidence for the ingestion and uptake of metals from a heterogeneous assortment of APPs in the field and under varying but natural, ambient conditions. Accordingly, the aims of the present study were to investigate the abundance of APPs in a variety of estuarine sediments and determine whether a keystone benthic organism, the harbour ragworm, *Hediste diversicolor*, is able to ingest APPs and accumulate biocidal metals under environmental conditions. *H. diversicolor* is a common, soft-bottom deposit- and filter-feeding polychaete that is widely distributed in estuaries within North West Europe and has an important ecosystem role as a bioturbator and a food source for numerous species of wading birds and flatfish (Goss-Custard et al., 1989; Budd, 2008). Intertidal sediment cores and *H. diversicolor* samples were collected around potential sources of antifouling waste, boating activity and control sites, and analysed for APPs and metals.

2. Methods

2.1. Study areas

In order to quantify the environmental concentrations of APPs, two estuaries in southwest UK were selected (Figure 1). The Plym is a small, macrotidal urban estuary located to the east of the city of Plymouth, with the upper reaches of the southern shores encompassing protected parkland and the lower estuary characterised by a high density of recreational boating activities, including marinas, boatyards and sailing clubs. The Erme is a small, macrotidal estuary located about 17 km to the south east of Plymouth, and is designated as an Area of Outstanding Natural Beauty (AONB) and a Site of Special Scientific Interest (SSSI) with no significant population, industries or boating activities within its catchment.

A total of eight sites were sampled within the two estuaries. Thus, a control site was selected in the Erme estuary (Wonwell), and seven sites were selected within the Plym estuary that comprised four sites located close to marinas and boatyards (University Marine Centre, Mount Batten, Hooe Point, Pomphlett Creek), one site around a mixed industrial area (Industrial), one site containing an abundance of old, abandoned boats (Hooe Lake), and one more protected site with minimal boating activity (Saltram Park).

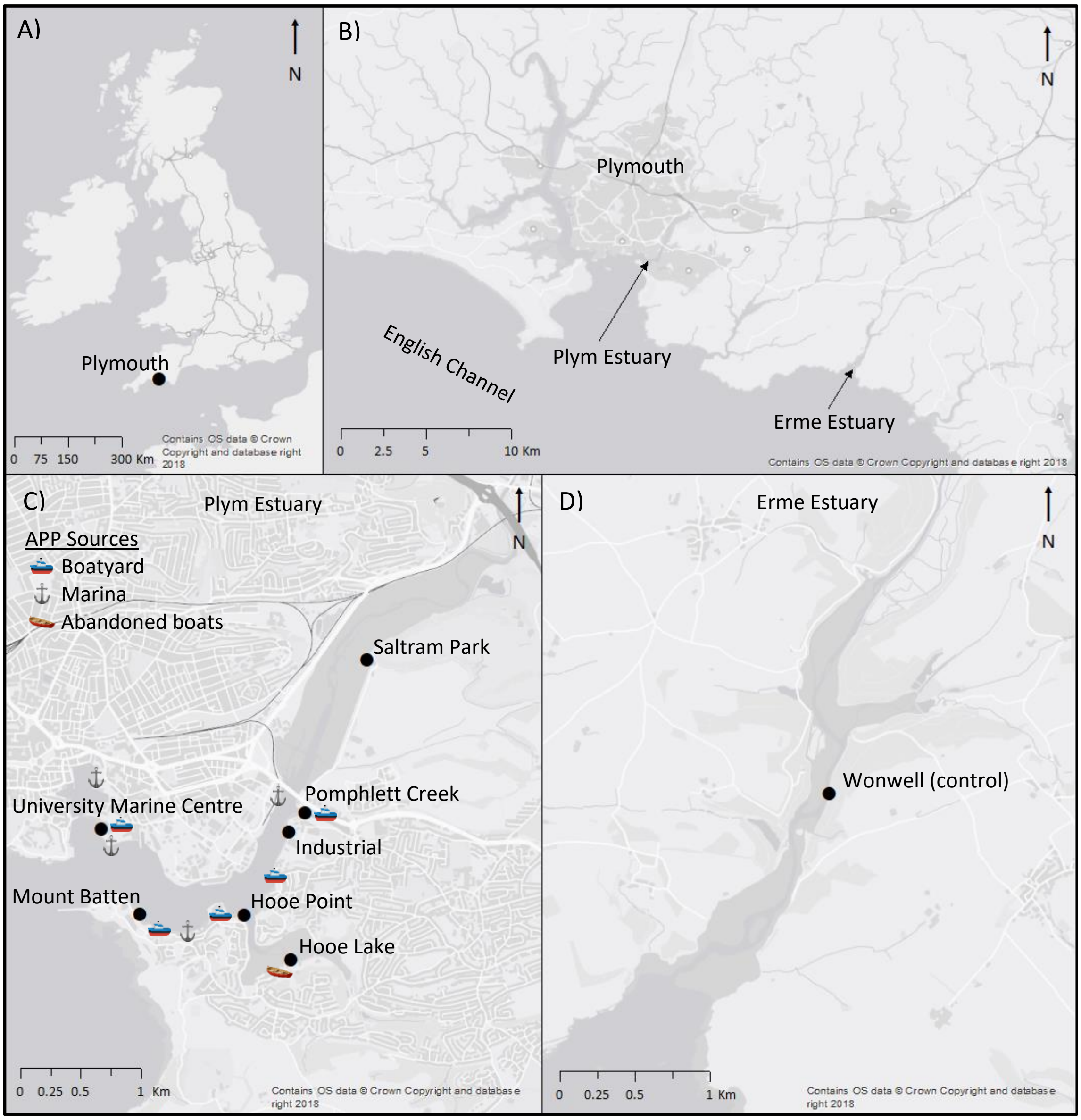


Figure 1. Location of Plymouth in the southwest of the UK (A), location of the Plym and Erme estuaries in relation to Plymouth (B), sampling locations in the Plym estuary in relation to potential sources of APPs (C) and the control site in the Erme estuary (D).

2.2 Sediment and *H. diversicolor* sampling

Sediment and *H. diversicolor* sampling was carried out once at each site at low tide during March and April 2018. Five randomised samples of silty, intertidal sediment, typically inhabited by *H. diversicolor*, were collected at each sampling location. Sediment samples were collected using a 5.1 cm diameter PVC hand core to a depth of approximately 20 cm, since *H. diversicolor* inhabit semi-permanent U or J-shaped burrows down to this depth (Budd, 2008). Samples were stored at 4 °C in the dark pending processing and analysis. *H. diversicolor* (around 5 - 10 cm in size) were collected *ad libitum* at all sites, except Mount Batten where this species was not evident, and transported back to the laboratory.

2.3 Sediment sample processing

Sediment samples were analysed for suspected APPs following a method similar to Soroldoni et al. (2018b). Thus, single 50 mL subsamples of the five samples from each site were weighed and fractionated through a series of stainless steel sieves (2 mm, 1 mm and 500 μm) with the aid of tap water. Material remaining on the sieves was transferred into a series of individual Petri dishes and systematically examined using a stereo microscope (Olympus SZX16). Suspected APPs were isolated, weighed on a five figure Sartorius balance, measured for primary diameter using a reticule ruler on the microscope, photographed, and their number and concentrations per litre of wet sediment and per kilogramme of dry sediment calculated.

2.4 APP metal analysis

Subsamples (~10%) of suspected APPs isolated from the sediment samples and at least 1 mm in diameter were analysed for the principal (heavy) metals that are, or that have been, used as biocides in antifouling formulations: Cu, Zn, Sn and Pb; using a battery-powered Niton X-ray fluorescence (XRF) spectrometer (model XL3t 950 He GOLDD+) housed in an accessory stand. Results from this non-destructive approach correlate well with those derived indirectly from paint particle acid digestion and analysis by inductively coupled plasma (ICP) spectrometry (Turner et al., 2014). Individual APPs were placed on laboratory grade Mylar film and positioned centrally over a 3 mm small-spot collimator above the detector. Samples were counted for 60 s in a low-density plastics mode coupled with thickness correction, with sample thickness measured using digital callipers. Two reference plastic discs certified for various metal concentrations were analysed before and after each batch of samples for instrument performance and quality assurance purposes. Limits of detection varied in accordance with sample composition, size and thickness but for particles > 2 mm in diameter were about 200 mg kg^{-1} for Pb and Zn and several hundred mg kg^{-1} for Cu and Sn.

Following metal analysis by XRF, suspected APPs were classified as either APP, based on the presence of Cu and/or Sn (at any concentration), or unknown, based on the absence of these elements. The latter classification includes alternative (non-antifouling) paints containing different elements, and including Zn and Pb (that have uses in various formulations other than as biocides), and relatively dense plastics that often contain metallic additives. The concentration and number of suspected APPs in sediments was subsequently corrected to true APPs by applying the percentage of confirmed APPs at each site.

Subsamples of <500 μm sediment were also analysed for metals by XRF. Here, material was dried at 60°C, homogenised using a pestle and mortar and stored in individual, clear polyethylene zip-bags. Five readings were taken at different locations with respect to the bagged contents for a period of 50 s using a higher density mining mode. Certified reference sediments were measured after every 20 readings for quality assurance purposes.

2.5 *H. diversicolor* sample processing and APP analysis

H. diversicolor were rinsed in tap water to remove external sediment, placed in falcon tubes and frozen overnight. Worms were then placed in an incubator at 50 °C for 1-2 days before dry weight was determined (1.41 $\underline{+}$ 0.93 g; $n = 19$). In order to digest tissue and isolate gut solids, 50 ml of 2 % technical grade potassium hydroxide was added to each tube and the contents incubated at 50 °C, with on/off shaking, for 6 d. Samples were then vacuum-filtered through a 35 µm nylon mesh, with KOH digests retained for metal analysis and residual material examined under a stereo microscope for suspected APPs which were subsequently isolated, weighed and photographed. Procedural blanks were prepared likewise but in the absence of worms.

Since particles retrieved from ragworm guts were too small for XRF analysis, suspected APPs were weighed into Eppendorf tubes and digested in 0.1 ml concentrated nitric acid (Fisher Trace Analysis Grade) for 1 h. Digests were diluted to 0.5 ml with distilled water and analysed for Cu, Zn, Sn and Pb by inductively coupled plasma mass spectrometry (ICP-MS) using a Thermo Scientific iCAP RQ. The instrument was calibrated with mixed standards (up to 2 mg L^{-1}) prepared by serial dilution of LabKings multi-element quality control solutions and an SCP Science single element calibration solution (Sn) in 2% HNO_3, and was operated under conditions described elsewhere (Turner, 2019). Based on the XRF measurements of suspected APPs above and the detection limits of the XRF, APPs were defined here as having a Cu concentration >200 mg kg^{-1} and/or a Sn concentration >100 mg kg^{-1}.

The filtered KOH digests were analysed for Cu (at 324.754 nm) and Zn (at 213.856 nm) by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Thermo Scientific iCAP 7000 Series ICP Spectrometer. The instrument was calibrated using mixed standards (up to 2 mg L^{-1}) prepared as above and was operated under conditions described elsewhere (Turner, 2019). Tin and Pb were analysed by ICP-MS following methods outlined above and with mixed, matrix-matched standards for calibration.

2.6 Statistical analysis

Statistical analyses were performed in R-Studio with an α value for significance of 0.05. The number and concentration of APPs in sediment were compared between sites using a Kruskal-Wallis test with Nemenyi's post-hoc test since these data were not normally distributed. Linear models were used to analyse the relationships between the different metal concentrations in APPs, metal concentrations in ragworms and sediment, and metal concentrations in *H. diversicolor* and APPs. Analysis of variance (ANOVA) with Tukey's post-hoc test was used to compare mean metal concentrations in worms between sites.

3. Results

3.1 APP abundance in sediment

Within the constraints of detection by microscopy, a total of 724 suspected APPs were isolated from the five replicate sediment samples examined from each site, with particles present at all locations with the exception of the control site (Wonwell) on the Erme estuary (Table 1). Individual particle mass ranged from < 1 mg to nearly 100 mg and the majority of suspected APPs were in the size range 500 µm – 1 mm with progressively fewer observed with increasing particle diamater. More than 100 suspected APPs were counted at Hooe Lake, the University Marine Centre and Mount Batten. XRF analyses of 71 particles from the different sampling sites confirmed that, overall, 28% were APPs based on their Cu or Sn content while 72% were of unknown origin and characteristics (including non-antifouling paints and various microplastics). Applying site-specific corrections to the data, Table 1 also shows the average number and mass of confirmed APPs per litre of fresh sediment and per kilogramme of dry sediment for each site. Thus, APPs were most abundant on both a mass and number basis in sediment samples from Hooe Lake (about 400 L^{-1} or 4 g L^{-1}), in the vicinity of several abandoned boats, and from the University Marine Station (about 430 L^{-1} or 0.24 g L^{-1}), where contemporary leisure boat maintenance takes place; APPs were not evident in sediments from Saltram Park, a region on the Plym estuary remote from any boating activities, the mixed industrial site on the Plym estuary, and the control site.

3.2 Metal concentrations in sediment APP

Table 2 shows the biocidal metal concentrations in a selection of APPs from each sampling site; note that detection limits increase with decreasing sample size, and that particles < 1 mm in diameter were not considered because of such a constraint on metal detection. Note also that other elements detected in some samples and at concentrations up to several thousand mg kg^{-1} (but not discussed further) included As, Ba, Cr and Hg. Concentrations of both Cu and Zn ranged from a few hundred mg kg^{-1} to more than 25% on a weight basis among the samples and concentrations of the two metals exhibited a significant correlation (r = 0.816). Tin was detected in six samples encompassing three sites, with one of the two highest concentrations associated with high concentrations of Cu and Zn and the other associated with no detectable

Cu and Zn. Lead was detected in nine samples, six of which were taken from Hooe Lake and adjacent to abandoned boats, and the metal was usually present where concentrations of Cu and Zn were relatively low.

Table 1. Total number of suspected APPs in the different size fractions of five replicate sediment samples from each site and, after correction based on selected chemical analyses, the average and maximum number and average and maximum mass of confirmed APPs per litre and per kilogramme of dry sediment.

	suspected APPs				confirmed APPs		
Site	500 µm - 1 mm	1 - 2 mm	> 2 mm	total	average (max) no. L^{-1}	average (max) g L^{-1}	average (max) g kg^{-1}
Hooe Lake	136	36	12	184	396 (722)	4.19 (18.8)	3.06 (13.7)
University Marine Centre	238	46	5	289	434 (788)	0.24 (0.53)	0.16 (0.36)
Mount Batten	120	18	1	139	79.5 (126)	0.02 (0.02)	0.01 (0.01)
Hooe Point	43	5	4	52	59.4 (91.4)	0.03 (0.06)	0.02 (0.04)
Pomphlett Creek	30	3	1	34	17.0 (22.5)	<0.01	< 0.01
Industrial	14	6	1	21	0	0	0
Saltram Park	1	0	1	2	0	0	0
Wonwell (control)	0	0	0	0	0	0	0

Table 2. Metal concentrations determined by XRF (mg kg^{-1}) in individual APPs isolated from sediment samples. Detection limits are shown as upper limits where metal concentrations were not returned by the instrument and vary according to sample composition, size and thickness.

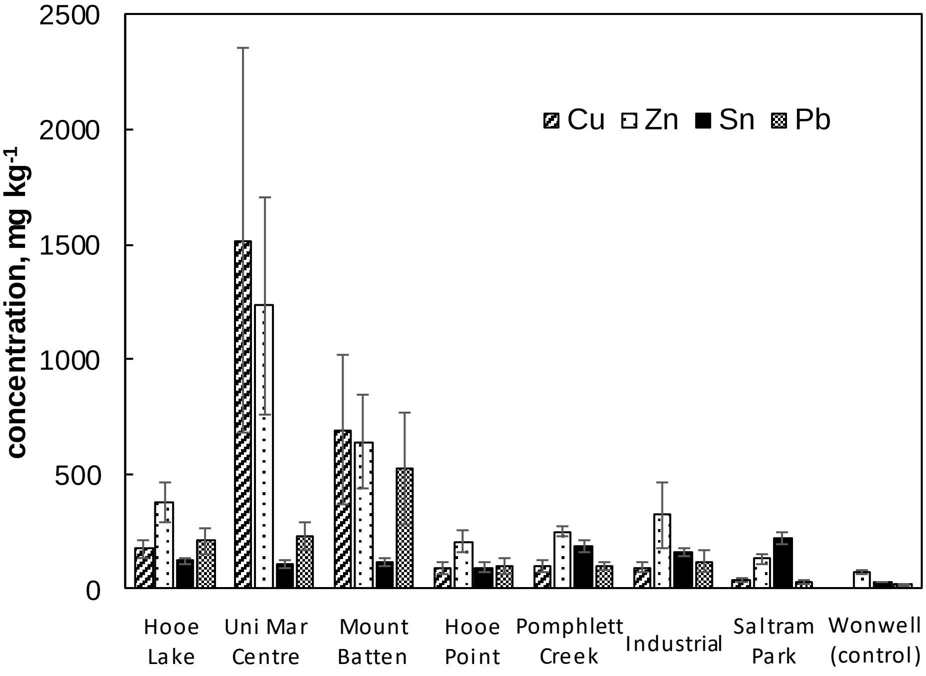
Site	APP diameter, mm	Cu	Zn	Sn	Pb
Hooe Lake	1–2	1570	2670	<1270	2760
	>2	19300	64500	<369	374
	>2	333	361	180	8810
	>2	<440	1550	127	408
	>2	708	<180	594	<42
	>2	1070	<192	366	<133
	>2	1980	815	<325	126000
University Marine Centre	1–2	562000	269000	7350	<2970
	1–2	540	32800	<384	5120
	1–2	224000	348000	<1620	<1380
	1–2	1590	989	<857	<76
	1–2	2850	2930	<598	<683
	1–2	1830	2400	<645	<399
	>2	221	196	<335	<31
	>2	2810	1210	<740	<62
	>2	6410	1130	<518	<186
Mount Batten	1–2	<2110	<1530	9970	5050
Hooe Point	1–2	1150	<590	<1230	<398
	>2	275	343	<267	221
Pomphlett Creek	1–2	1420	<348	<633	<96

3.3. Metal concentrations in sediment

The concentrations of Cu and Zn in the <500 μm sediment fraction and as determined by XRF are shown in Figure 2. Here, mean values arising from multiple measurements of replicate subsamples are reported, with concentration variations therefore reflecting heterogeneity both within and between sample sites. Mean concentrations of Cu were greatest at the University Marine Centre (about 1500 mg kg^{-1}), with individual measurements at this site ranging from about 800 to over 3100 mg kg^{-1}. Copper was never detected in samples from the control site (with a detection limit of about 25 mg kg^{-1}) and averaged about 35 mg kg^{-1} at Saltram Park. Mean concentrations of Zn ranged from about 70 mg kg^{-1} at the control site to 1200 mg kg^{-1} at the University Marine Centre, with individual concentrations at the latter site ranging from about 700 to 2000 mg kg^{-1}.

Mean concentrations of Pb ranged from about 17 mg kg^{-1} at the control site to > 500 mg kg^{-1} at Mount Batten, with the highest individual concentration returned close to 1200 mg kg^{-1}. Tin was only detected in three replicates from the control site (and at about 30 mg kg^{-1}) and averaged between 100 and 200 mg kg^{-1} at remaining sites in the Plym estuary.

Figure 2: Mean metal concentrations in the < 500 μm sediment fraction for each sampling site and as determined by XRF. Errors represent one standard deviation.



3.4. APPs and metals in *H. diversicolor*

A total of 44 suspected APPs were retrieved from the guts of 11 individuals of *H. diversicolor* from five sites. ICP-MS analysis of 36 particles (of sufficient size) confirmed that 16 (or 45%) from five individuals and from three sites that ranged from about 100 μm to 2.6 mm in diameter were APPs (Table 3). The majority of APPs were found in worms from the University Marine Centre and with a range of metal concentrations that is broadly consistent with corresponding ranges reported for sediment APPs in Table 2; note, however, that ICP-MS is more sensitive than XRF and therefore extends the lower end of the concentration range for each metal. Individual APPs were also encountered in animal guts from Hooe Lake and from Hooe Point.

The concentrations of metals in the tissues of *H. diversicolor* are shown in Table 4. Individual concentrations of Cu ranged from 3.6 to 74.7 mg kg^{-1} and mean concentrations were significantly different across the sites. Specifically, the highest mean ragworm Cu concentration was found at the University Marine Centre (59.9 ± 23.6 mg kg^{-1}) with high concentrations also observed in individuals originating from Hooe Point and Hooe Lake; significantly, these were the only sites where APPs were confirmed in the animal guts. The lowest mean Cu concentrations were encountered at Saltram Park (4.27 ± 0.71 mg kg^{-1}) and the control site (9.54 ± 4.07 mg kg^{-1}), and a significant linear relationship was observed between Cu concentrations in ragworms and mean Cu concentrations in < 500 μm sediment fraction (Figure 3).

Table 3. Metal concentrations determined by ICP-MS (mg kg^{-1}) in 16 individual APPs isolated from samples of *H. diversicolor* from three sites. Note the prevalence of APPs at the University Marine Centre.

Site	Cu	Zn	Sn	Pb
Hooe Lake	690	1240	1.70	98.5
University Marine Centre	500	118	2.60	3.83
	16400	6040	24.3	59.8
	16300	388	12.2	34.3
	2190	715	20.9	31.6
	673	1220	7.49	9.26
	286	23.9	19.78	6.29
	3430	1040	41.1	133
	511	147	3.36	12.4
	3520	12200	52.4	107
	17200	49500	5490	79.7
	335	290	15.0	7.47
	292	434	58.4	1.16
	337	240	3.92	6.68
	2994	289	9.44	22.2
Hooe Point	407	1240	31.4	75.8

Table 4: Tissue concentrations of metals (in mg kg^{-1}) in individuals of *H. diversicolor*. The number of individuals from each location is shown (*n*) along with individual concentrations (*n* = 2) or the concentration mean ± one sd (*n* = 3).

site	Cu	Zn	Sn	Pb
Hooe Lake (*n* = 2)	15.7; 26.8	70.6;57.2	1.34; 0.72	12.6; 10.7
University Marine Centre (*n* = 3)	59.9±23.6	69.9±7.00	3.35±0.66	4.24±1.33
Hooe Point (*n* = 2)	19.3; 54.6	26.0; 35.7	1.27; 0.98	11.7; 20.7
Pomphlett Creek (*n* = 3)	13.6±2.61	25.6±5.43	1.04±0.17	6.07±1.55
Industrial (*n* = 3)	10.1±2.98	45.5±5.55	0.63±0.06	5.67±0.63
Saltram Park (*n* = 3)	4.27±0.71	28.3±6.36	0.91±0.04	3.53±0.32
Wonwell (control) (*n* = 3)	9.54±4.07	46.2±10.9	0.22±0.19	5.33±1.21

Tissue concentrations of Zn in individuals of *H. diversicolor* ranged from 21.5 to 77.5 mg kg^{-1} with mean concentrations significantly greater at the University Marine Centre (69.9 ± 7.00 mg kg^{-1}) and Hooe Lake (69.9 ± 9.48 mg kg^{-1}) than at remaining sites, including the control (46.2 ± 10.9 mg kg^{-1}). There was a significant linear relationship between Zn concentrations in worms and mean Zn concentrations in < 500 μm sediment (Figure 3). Moreover, there was a significant relationship between Cu and Zn concentrations in ragworm tissue ($r = 0.595$), albeit when the best-fit line was defined with an intercept of about 35 mg kg^{-1} through the Zn-axis.

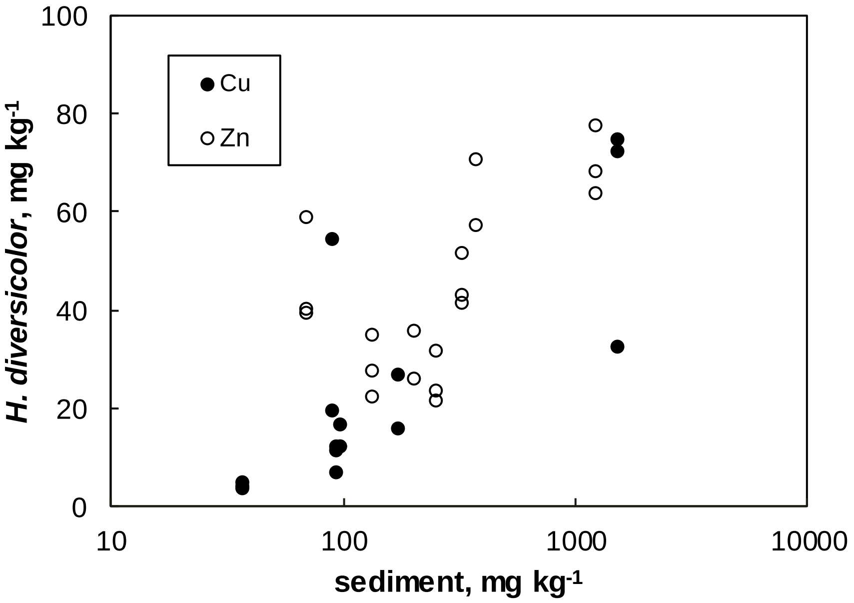


Figure 3: Relationships between metal concentrations in < 500 μm sediment and *H. diversicolor* tissue (● Cu, *r* = 0.792; o Zn, *r* = 0.635).

Tissue concentrations of Sn in individual *H. diversicolor* ranged from about 0.32 to 4.05 mg kg^{-1} with mean concentrations significantly greater at the University Marine Centre (3.35 $\pm$ 0.67 mg kg^{-1}) than at remaining sites, including the control (0.22 $\pm$ 0.19 mg kg^{-1}). Concentrations of Sn and Cu (but not Sn and Zn) in worms were significantly correlated (*r* = 0.785) but concentrations of Sn in sediment or APPs were not related to Sn concentrations in *H. diversicolor*. Tissue concentrations of Pb were less variable among individuals and sites than the other metals studied with concentrations that ranged from about 3 to 20 mg kg^{-1}. Average tissue Pb concentrations were significantly greater in animals from Hooe Lake and Hooe Point than in worms from the remaining sites but, overall, there was no relationship between Pb concentrations in tissue and in sediment.

4. Discussion

This study has demonstrated that APPs are abundant in intertidal sediment both near to contemporary boating activities and to older, abandoned boats. Of the anthropogenic particles isolated from the sediments, 30% were classed as APPs based on their Cu or Sn content, with the remainder likely to be non-antifouling paint or plastic particles. Polymer (alkyl ester) resins are a major constituent of solvent-based paints, and as such paint particles have been categorised as a type of secondary microplastic (microscopic plastic; MEPEX, 2013). Paint fragments have been identified in waterborne and sediment samples from across the globe, and recent estimates suggest APPs may be a substantial contributor to microplastic loads (EUNOMIA, 2016). The risks microplastics pose to marine ecosystems is under intense scrutiny, with particular concern that they may act as a 'Trojan horse' whereby their ingestion results in the direct transfer of toxic chemicals associated with the plastic (Syberg et al., 2015; Galloway et al., 2017). It is therefore particularly surprising that APPs have not received greater scientific and regulatory attention.

In the present study, APPs were highly heterogeneous in abundance, size and chemical makeup, both between and within different sites. However, overall the highest APP loads were encountered in the vicinity of current or historic boating activities, an observation consistent with a previous study demonstrating that biocidal APPs were localised to boating activity in a Brazilian estuary, with no evidence of transport towards the coastline (Sorolodini et al., 2018b). An increase in the number of APPs per L of sediment with decreasing particle size is similar to recent observations reported for the size distribution of pigmented and metal-rich microplastics in a limnetic system, an effect that was attributed to the occurrence and relative brittleness of paint particles among the plastic population (Imhof et al., 2016). The concentrations of biocidal metals (Cu, Zn, Sn and Pb) in the APPs varied by orders of magnitude, as found in a study of APP composition near boatyards (Turner, 2010) and abandoned boats (Rees et al., 2014), which reflects both the heterogeneity of formulations used over the years and their differential degrees of leaching (or ages since deposition).

An immediate impact of APPs is contamination of local sediment; this was evident for all metals except Sn which has a relatively high inorganic background signature because of the presence of primary deposits of cassiterite in the region (Thorndycraft et al., 2004). (Note that our analytical methods are unable to discriminate inorganic and organic tin.) Sediment contamination from APPs may take place directly through the presence of metal-rich particles, or indirectly through metal leaching into interstitial waters and subsequent adsorption to neighbouring sediment grains (Takahashi et al., 2012). Significant in this respect is the presence of historic paint particles that provide a localised, secondary source of biocides that are currently banned (including TBT and organolead compounds; Thomas et al., 2003; Rees et al., 2014; Largerström et al., 2017). In many cases, contamination leads to metal concentrations that exceed probable effects levels (where adverse effects frequently occur) in the marine environment, as defined by the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (Cu = 108 mg kg^{-1}; Zn = 271 mg kg^{-1}; Pb = 112 mg kg^{-1}; CCME, 2014).

Specifically, mean concentrations of Cu, Zn and Pb exceed their respective probable effects levels at the three sites where APPs were most abundant. As a caveat, however, it must be borne in mind that these and other relevant standards implicitly assume that the metals are present in inorganic, bivalent form, while in APPs Cu is present in univalent form as Cu_2O or Cu thiocyanate (Yebra et al., 2004) and other metals may be bound as specific biocidal, compounds (e.g. Zn pyrithione) whose toxicities are not well-defined.

Although previous laboratory studies have shown evidence for APP ingestion by benthic organisms (Gammon et al., 2009; Turner et al., 2009), this study is the first to directly observe the presence of APPs in the digestive system of an animal in its natural habitat. APPs were most prevalent in *H. diversicolor* inhabiting sediments impacted by local contemporary or historical boating activities and, as with APPs in sediment, they were highly heterogeneous in chemical makeup with metal concentrations spanning an order of magnitude or more in APPs from the same animal or in animals from the same site. This suggests that, at least for the organism under study, material is ingested non-selectively in terms of both particle type and chemical composition.

Elevated but heterogeneous concentrations of Cu, Zn, Sn and Pb were also evident in the tissue of *H. diversicolor* sampled from locations impacted by contemporary boating activity or in the vicinity of abandoned boats; elevated Pb concentrations are, however, restricted to the latter locations, consistent with the application of Pb in historic antifouling formulations and in older non-antifouling paints (used on boat cabins and decks, for example; Booher, 1988). Relative to tissue concentrations in the corresponding control samples, Cu exhibits considerably greater enrichment in animals from contaminated sites than Zn, despite broadly similar concentrations of these metals in contaminated sediments and in APPs. For example, at the University Marine Centre, mean Cu concentrations are six times higher than those at the control site whereas Zn concentrations are just 50 % greater. Accumulation of Cu in *H. diversicolor* has previously been linked to high environmental concentrations and there is evidence that the ragworm is unable to regulate Cu body concentrations in many locations, leading to bioaccumulation of the metal (Berthet et al., 2003). Conversely, Zn concentrations have been found to remain relatively constant in ragworms, regardless of external concentrations, with the animal appearing to regulate this metal to a certain extent, albeit by reducing energy reserves (Durou et al., 2005).

The widespread occurrence of Cu-rich APPs retrieved from the guts of individuals of *H. diversicolor* suggests that accumulation of the metal may, at least partly, take place through the solubilisation of the metal in the digestive environment and transfer to the circulatory fluids. An *in vitro* study undertaken by Jones and Turner (2010) demonstrated that both surfactants and enzymes typical of the gut fluids of marine deposit-feeding organisms are able to facilitate the mobilisation of Cu from APPs into sea water through a series of complexation and exchange reactions. However, the measurements of the present study are unable to discriminate this mode of uptake from absorption of Cu via interstitial waters enriched in APP leachate.

Regardless of the precise uptake mechanism/s involved, accumulation of Cu and other biocidal metals raises the possibility of toxic responses in the animal. It is well known that the accumulation of trace metals can have toxic effects on organisms and studies have shown that metals contained within APPs are toxic to marine organisms. Soroldoni et al. (2018c) found a significant reduction in fecundity and increase in mortality of the epibenthic copepod, *Nitokra* sp., with increasing concentrations of APPs in sediments, with Cu shown to have a higher toxicity than Zn. Ytreberg et al. (2010) found that APP leachate caused a reduction in *Vibrio fischeri* bioluminescence, decreased growth rate of *Ceramium tenuicorne* and reduced larval development in *N. spinipes*, with Cu more toxic to *V. fischeri* and *C. tenuicorne* and Zn more toxic to *Nitocra spinipes*. More general toxicity studies involving Cu have also shown that exposure to the metal can exert a variety of effects on *H. diversicolor,* including increased mortality and reduced burrowing activity (Thit et al, 2015). Given the exceptionally high Cu concentrations contained within APPs and the ecological importance of ragworms in estuarine sediments, contamination by antifouling waste could have significant implications for ecosystem functioning.

In conclusion, this study has shown that high quantities of APP enriched in one or more biocidal metal (Cu, Zn, Sn, Pb) occur in sediments where boats are maintained or, after abandonment, weathered. APPs have been identified in the digestive tracts of an important benthic organism, *H. diversicolor*, with concentrations of Cu in the animal reflecting concentrations in local contaminated sediment and suggesting that the organism is not able to fully regulate the metal. With impacts on both estuarine sediment quality and, potentially, organism and ecosystem health, tighter controls on the management or disposal of contemporary antifouling paint wastes and the safeguarding of historical paints are called for.

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