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

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

by

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### 53 **Abstract**

54 Antifouling paint particles (APPs) of between 500 µm and > 2 mm in diameter have been  
55 identified in silty, intertidal estuarine sediments through a combination of microscopy and x-  
56 ray fluorescence spectrometry. APPs were heterogeneously distributed, with maximal  
57 concentrations of 430 particles L<sup>-1</sup> (0.2 g L<sup>-1</sup>) near to a facility where boats are regularly  
58 maintained and 400 particles L<sup>-1</sup> (4.2 g L<sup>-1</sup>) at a location where old boats had been abandoned,  
59 with the majority of particles encountered in the finest size fraction retrieved. APPs contained  
60 variable concentrations of Cu, Zn, Sn and Pb, with respective maxima of 562,000, 269,000,  
61 9,970 and 126,000 mg kg<sup>-1</sup>. These characteristics are attributed to a multitude of  
62 contemporary and historic sources of an assortment of formulations and result in significant  
63 but heterogeneous metal contamination of local sediments. APPs were also identified in the  
64 guts of the deposit-feeding ragworm, *Hediste diversicolor*, that inhabited sediments impacted  
65 by abandoned boats or boating activities. The tissue of *H. diversicolor* was particularly enriched  
66 in Cu where ingested APPs were observed, with a significant correlation between Cu  
67 concentrations in the two media ( $r = 0.734$ ) presumably reflecting the inability of the animal to  
68 regulate this metal. While the toxicity of APPs requires further investigation, there is clearly a  
69 need for stricter regulations on antifouling wastes in boatyards and marinas and a requirement  
70 to better manage abandoned boats.

71

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

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

76

### 77 **1. Introduction**

78 Marine biofouling is caused by the accumulation of unwanted organisms on submerged  
79 structures. Adverse impacts of biofouling include additional cleaning and maintenance costs  
80 and increased frictional drag on boats, leading to higher fuel consumption and reduced  
81 manoeuvrability (Chambers et al., 2006). Antifouling paint is applied to many marine  
82 structures to reduce biofouling and typically works by leaching or eroding biocides into the

83 surrounding environment (Yebra et al., 2004). Organotin-based formulations were popular  
84 antifoulants for several decades until significant, adverse effects on non-target species were  
85 demonstrated (Bailey and Davies, 1991; Axiak et al., 2000). The subsequent phasing out of  
86 antifouling paints containing organotins resulted in the development of new, tin-free paints,  
87 with most contemporary antifouling paints containing Cu(I) as the main biocide, often in  
88 combination with Zn-based compounds such as zinc oxide (ZnO), and a combination of booster  
89 biocides that include zinc pyrithione (ZnPT), Irgarol 1051 and diuron (Chambers et al., 2006).

90

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

107

108 Benthic organisms are essential for the functioning of marine coastal ecosystems and play an  
109 important role in energy transfer between pelagic and benthic ecosystems. Controlled  
110 laboratory studies have shown that exposure to APPs can lead to the accumulation of the  
111 biocidal metals, Cu and Zn, in the tissues of the common mussel *Mytilus edulis* (Turner et al.,  
112 2009), the common periwinkle *Littorina littorea* (Gammon et al., 2009) and the lugworm  
113 *Arenicola marina* (Turner et al., 2008b). Uptake of metals appears to take place through both  
114 aqueous exposure to APP leachate or via direct ingestion of APPs, with organisms unable to  
115 differentiate APPs from their food. What has been lacking, however, is evidence for the  
116 ingestion and uptake of metals from a heterogeneous assortment of APPs in the field and  
117 under varying but natural, ambient conditions. Accordingly, the aims of the present study were  
118 to investigate the abundance of APPs in a variety of estuarine sediments and determine

119 whether a keystone benthic organism, the harbour ragworm, *Hediste diversicolor*, is able to  
120 ingest APPs and accumulate biocidal metals under environmental conditions. *H. diversicolor* is  
121 a common, soft-bottom deposit- and filter-feeding polychaete that is widely distributed in  
122 estuaries within North West Europe and has an important ecosystem role as a bioturbator and  
123 a food source for numerous species of wading birds and flatfish (Goss-Custard et al., 1989;  
124 Budd, 2008). Intertidal sediment cores and *H. diversicolor* samples were collected around  
125 potential sources of antifouling waste, boating activity and control sites, and analysed for APPs  
126 and metals.

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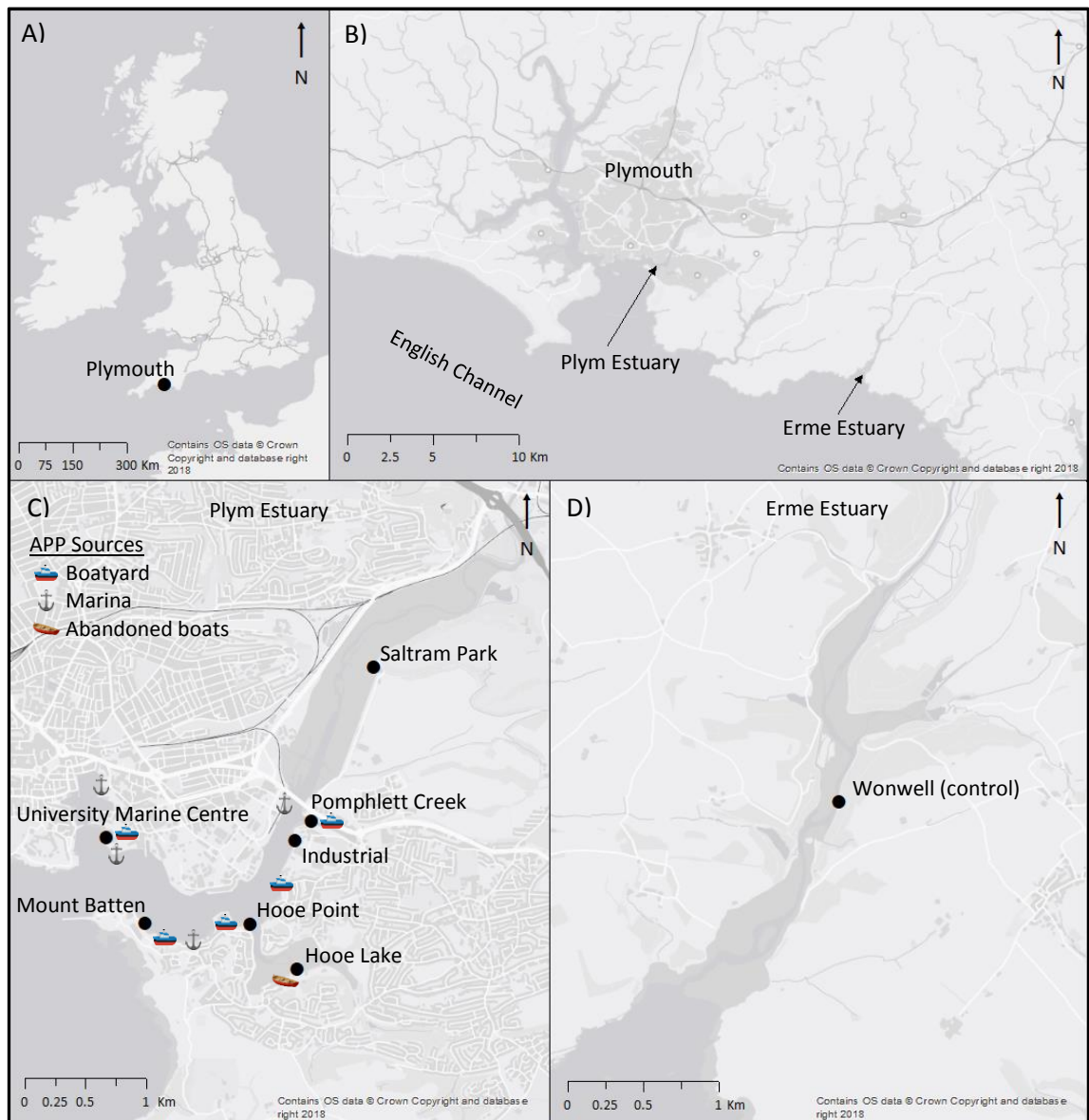
## 128 **2. Methods**

### 129 **2.1. Study areas**

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

138

139 A total of eight sites were sampled within the two estuaries. Thus, a control site was selected  
140 in the Erme estuary (Wonwell), and seven sites were selected within the Plym estuary that  
141 comprised four sites located close to marinas and boatyards (University Marine Centre, Mount  
142 Batten, Hooe Point, Pomphlett Creek), one site around a mixed industrial area (Industrial), one  
143 site containing an abundance of old, abandoned boats (Hooe Lake), and one more protected  
144 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).

145 **2.2 Sediment and *H. diversicolor* sampling**

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

154 **2.3 Sediment sample processing**

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

163 **2.4 APP metal analysis**

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

178

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

186

187 Subsamples of <500  $\mu\text{m}$  sediment were also analysed for metals by XRF. Here, material was  
188 dried at 60°C, homogenised using a pestle and mortar and stored in individual, clear

189 polyethylene zip-bags. Five readings were taken at different locations with respect to the  
190 bagged contents for a period of 50 s using a higher density mining mode. Certified reference  
191 sediments were measured after every 20 readings for quality assurance purposes.

## 192 **2.5 *H. diversicolor* sample processing and APP analysis**

193 *H. diversicolor* were rinsed in tap water to remove external sediment, placed in falcon tubes  
194 and frozen overnight. Worms were then placed in an incubator at 50 °C for 1-2 days before dry  
195 weight was determined ( $1.41 \pm 0.93$  g;  $n = 19$ ). In order to digest tissue and isolate gut solids,  
196 50 ml of 2 % technical grade potassium hydroxide was added to each tube and the contents  
197 incubated at 50 °C, with on/off shaking, for 6 d. Samples were then vacuum-filtered through a  
198 35  $\mu$ m nylon mesh, with KOH digests retained for metal analysis and residual material  
199 examined under a stereo microscope for suspected APPs which were subsequently isolated,  
200 weighed and photographed. Procedural blanks were prepared likewise but in the absence of  
201 worms.

202

203 Since particles retrieved from ragworm guts were too small for XRF analysis, suspected APPs  
204 were weighed into Eppendorf tubes and digested in 0.1 ml concentrated nitric acid (Fisher  
205 Trace Analysis Grade) for 1 h. Digests were diluted to 0.5 ml with distilled water and analysed  
206 for Cu, Zn, Sn and Pb by inductively coupled plasma mass spectrometry (ICP-MS) using a  
207 Thermo Scientific iCAP RQ. The instrument was calibrated with mixed standards (up to 2 mg L<sup>-1</sup>  
208 <sup>1</sup>) prepared by serial dilution of LabKings multi-element quality control solutions and an SCP  
209 Science single element calibration solution (Sn) in 2% HNO<sub>3</sub>, and was operated under  
210 conditions described elsewhere (Turner, 2019). Based on the XRF measurements of suspected  
211 APPs above and the detection limits of the XRF, APPs were defined here as having a Cu  
212 concentration >200 mg kg<sup>-1</sup> and/or a Sn concentration >100 mg kg<sup>-1</sup>.

213

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

## 220 **2.6 Statistical analysis**

221 Statistical analyses were performed in R-Studio with an  $\alpha$  value for significance of 0.05. The  
222 number and concentration of APPs in sediment were compared between sites using a Kruskal-  
223 Wallis test with Nemenyi's post-hoc test since these data were not normally distributed. Linear



224 models were used to analyse the relationships between the different metal concentrations in  
225 APPs, metal concentrations in ragworms and sediment, and metal concentrations in *H.*  
226 *diversicolor* and APPs. Analysis of variance (ANOVA) with Tukey's post-hoc test was used to  
227 compare mean metal concentrations in worms between sites.

228

### 229 **3. Results**

#### 230 **3.1 APP abundance in sediment**

231 Within the constraints of detection by microscopy, a total of 724 suspected APPs were isolated  
232 from the five replicate sediment samples examined from each site, with particles present at all  
233 locations with the exception of the control site (Wonwell) on the Erme estuary (Table 1).

234 Individual particle mass ranged from < 1 mg to nearly 100 mg and the majority of suspected  
235 APPs were in the size range 500 µm – 1 mm with progressively fewer observed with increasing  
236 particle diameter. More than 100 suspected APPs were counted at Hooe Lake, the University  
237 Marine Centre and Mount Batten. XRF analyses of 71 particles from the different sampling  
238 sites confirmed that, overall, 28% were APPs based on their Cu or Sn content while 72% were  
239 of unknown origin and characteristics (including non-antifouling paints and various  
240 microplastics). Applying site-specific corrections to the data, Table 1 also shows the average  
241 number and mass of confirmed APPs per litre of fresh sediment and per kilogramme of dry  
242 sediment for each site. Thus, APPs were most abundant on both a mass and number basis in  
243 sediment samples from Hooe Lake (about 400 L<sup>-1</sup> or 4 g L<sup>-1</sup>), in the vicinity of several  
244 abandoned boats, and from the University Marine Station (about 430 L<sup>-1</sup> or 0.24 g L<sup>-1</sup>), where  
245 contemporary leisure boat maintenance takes place; APPs were not evident in sediments from  
246 Saltram Park, a region on the Plym estuary remote from any boating activities, the mixed  
247 industrial site on the Plym estuary, and the control site.

#### 248 **3.2 Metal concentrations in sediment APP**

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

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

261

262

**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.

263

Site	suspected APPs				confirmed APPs		
	500 $\mu\text{m}$ - 1 mm	1 - 2 mm	> 2 mm	total	average (max) no. L <sup>-1</sup>	average (max) g L <sup>-1</sup>	average (max) g kg <sup>-1</sup>
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
264 Wonwell (control)	0	0	0	0	0	0	0

265 **Table 2.** Metal concentrations determined by XRF (mg kg<sup>-1</sup>) in individual APPs isolated from  
 266 sediment samples. Detection limits are shown as upper limits where metal concentrations  
 267 were not returned by the instrument and vary according to sample composition, size and  
 268 thickness.

269

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
Mount Batten	>2	6410	1130	<518	<186
	1-2	<2110	<1530	9970	5050
	1-2	1150	<590	<1230	<398
Hooe Point	>2	275	343	<267	221
	1-2	1420	<348	<633	<96
Pomphlett Creek	1-2	1420	<348	<633	<96

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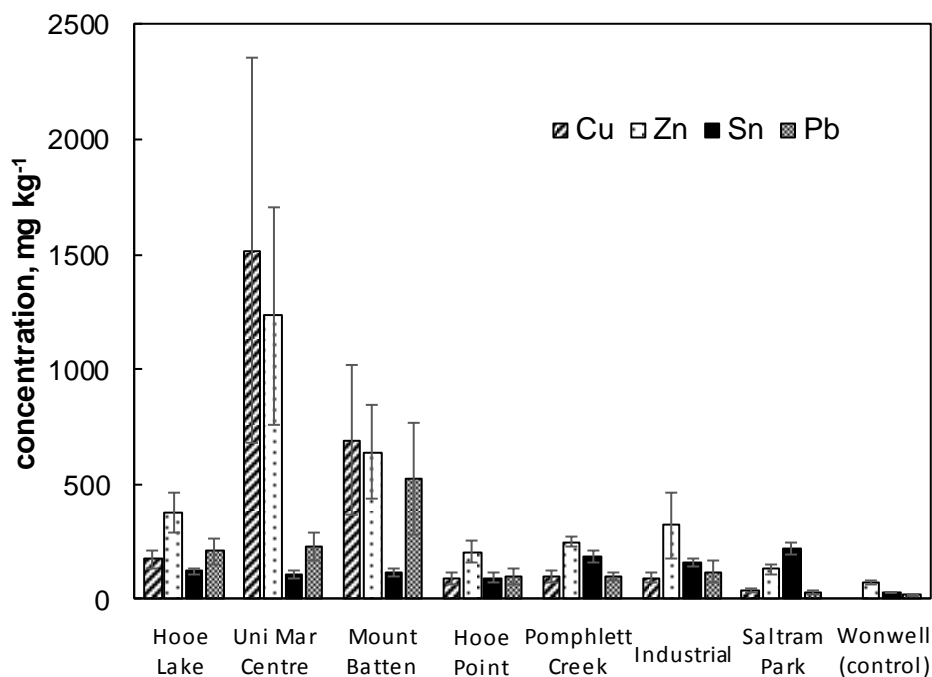
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273 **3.3. Metal concentrations in sediment**

274 The concentrations of Cu and Zn in the <500  $\mu\text{m}$  sediment fraction and as determined by XRF  
275 are shown in Figure 2. Here, mean values arising from multiple measurements of replicate  
276 subsamples are reported, with concentration variations therefore reflecting heterogeneity  
277 both within and between sample sites. Mean concentrations of Cu were greatest at the  
278 University Marine Centre (about  $1500 \text{ mg kg}^{-1}$ ), with individual measurements at this site  
279 ranging from about 800 to over  $3100 \text{ mg kg}^{-1}$ . Copper was never detected in samples from the  
280 control site (with a detection limit of about  $25 \text{ mg kg}^{-1}$ ) and averaged about  $35 \text{ mg kg}^{-1}$  at  
281 Saltram Park. Mean concentrations of Zn ranged from about  $70 \text{ mg kg}^{-1}$  at the control site to  
282  $1200 \text{ mg kg}^{-1}$  at the University Marine Centre, with individual concentrations at the latter site  
283 ranging from about 700 to  $2000 \text{ mg kg}^{-1}$ .

284 Mean concentrations of Pb ranged from about  $17 \text{ mg kg}^{-1}$  at the control site to  $> 500 \text{ mg kg}^{-1}$  at  
285 Mount Batten, with the highest individual concentration returned close to  $1200 \text{ mg kg}^{-1}$ . Tin  
286 was only detected in three replicates from the control site (and at about  $30 \text{ mg kg}^{-1}$ ) and  
287 averaged between 100 and  $200 \text{ mg kg}^{-1}$  at remaining sites in the Plym estuary.  
288

289 **Figure 2:** Mean metal concentrations in the < 500  $\mu\text{m}$  sediment fraction for each sampling site  
290 and as determined by XRF. Errors represent one standard deviation.



291

292

293 **3.4. APPs and metals in *H. diversicolor***

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

303

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

314

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

315

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

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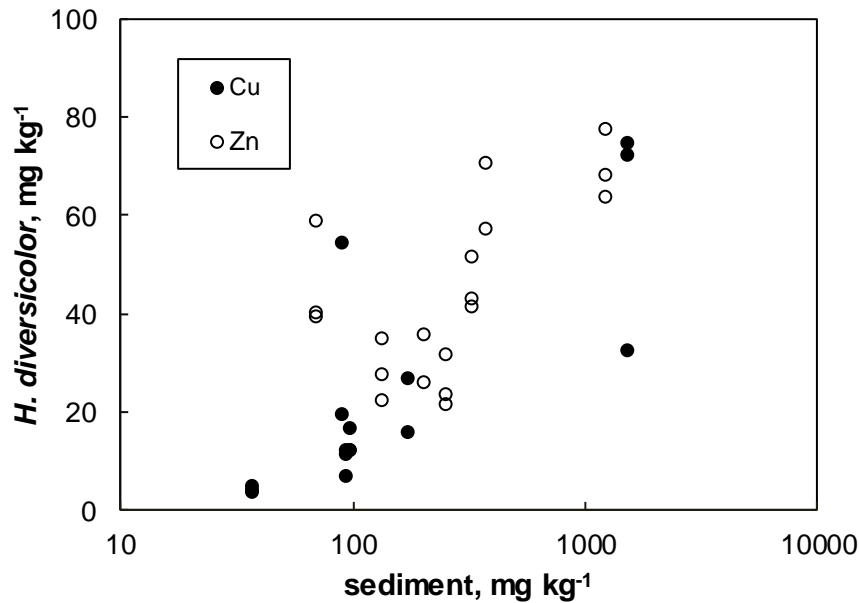
318 **Table 4:** Tissue concentrations of metals (in mg kg<sup>-1</sup>) in individuals of *H. diversicolor*. The  
319 number of individuals from each location is shown (*n*) along with individual concentrations (*n* =  
320 2) or the concentration mean  $\pm$  one sd (*n* = 3).

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

321

322

323 Tissue concentrations of Zn in individuals of *H. diversicolor* ranged from 21.5 to 77.5 mg kg<sup>-1</sup>  
324 with mean concentrations significantly greater at the University Marine Centre (69.9  $\pm$  7.00 mg  
325 kg<sup>-1</sup>) and Hooe Lake (69.9  $\pm$  9.48 mg kg<sup>-1</sup>) than at remaining sites, including the control (46.2  $\pm$   
326 10.9 mg kg<sup>-1</sup>). There was a significant linear relationship between Zn concentrations in worms  
327 and mean Zn concentrations in < 500  $\mu$ m sediment (Figure 3). Moreover, there was a  
328 significant relationship between Cu and Zn concentrations in ragworm tissue ( $r = 0.595$ ), albeit  
329 when the best-fit line was defined with an intercept of about 35 mg kg<sup>-1</sup> through the Zn-axis.



330

331

332 Figure 3: Relationships between metal concentrations in < 500 μm sediment and *H.*

333 *diversicolor* tissue (● Cu,  $r = 0.792$ ; ○ Zn,  $r = 0.635$ ).

334

335

336 Tissue concentrations of Sn in individual *H. diversicolor* ranged from about 0.32 to 4.05 mg kg<sup>-1</sup>

337 with mean concentrations significantly greater at the University Marine Centre ( $3.35 \pm 0.67$  mg

338 kg<sup>-1</sup>) than at remaining sites, including the control ( $0.22 \pm 0.19$  mg kg<sup>-1</sup>). Concentrations of Sn

339 and Cu (but not Sn and Zn) in worms were significantly correlated ( $r = 0.785$ ) but

340 concentrations of Sn in sediment or APPs were not related to Sn concentrations in *H.*

341 *diversicolor*. Tissue concentrations of Pb were less variable among individuals and sites than

342 the other metals studied with concentrations that ranged from about 3 to 20 mg kg<sup>-1</sup>. Average

343 tissue Pb concentrations were significantly greater in animals from Hooe Lake and Hooe Point

344 than in worms from the remaining sites but, overall, there was no relationship between Pb

345 concentrations in tissue and in sediment.

#### 346 4. Discussion

347

348 This study has demonstrated that APPs are abundant in intertidal sediment both near to

349 contemporary boating activities and to older, abandoned boats. Of the anthropogenic particles

350 isolated from the sediments, 30% were classed as APPs based on their Cu or Sn content, with

351 the remainder likely to be non-antifouling paint or plastic particles. Polymer (alkyl ester) resins

352 are a major constituent of solvent-based paints, and as such paint particles have been

353 categorised as a type of secondary microplastic (microscopic plastic; MEPEX, 2013). Paint  
354 fragments have been identified in waterborne and sediment samples from across the globe,  
355 and recent estimates suggest APPs may be a substantial contributor to microplastic loads  
356 (EUNOMIA, 2016). The risks microplastics pose to marine ecosystems is under intense scrutiny,  
357 with particular concern that they may act as a 'Trojan horse' whereby their ingestion results in  
358 the direct transfer of toxic chemicals associated with the plastic (Syberg et al., 2015; Galloway  
359 et al., 2017). It is therefore particularly surprising that APPs have not received greater scientific  
360 and regulatory attention.

361

362 In the present study, APPs were highly heterogeneous in abundance, size and chemical  
363 makeup, both between and within different sites. However, overall the highest APP loads were  
364 encountered in the vicinity of current or historic boating activities, an observation consistent  
365 with a previous study demonstrating that biocidal APPs were localised to boating activity in a  
366 Brazilian estuary, with no evidence of transport towards the coastline (Sorolodini et al.,  
367 2018b). An increase in the number of APPs per L of sediment with decreasing particle size is  
368 similar to recent observations reported for the size distribution of pigmented and metal-rich  
369 microplastics in a limnetic system, an effect that was attributed to the occurrence and relative  
370 brittleness of paint particles among the plastic population (Imhof et al., 2016). The  
371 concentrations of biocidal metals (Cu, Zn, Sn and Pb) in the APPs varied by orders of  
372 magnitude, as found in a study of APP composition near boatyards (Turner, 2010) and  
373 abandoned boats (Rees et al., 2014), which reflects both the heterogeneity of formulations  
374 used over the years and their differential degrees of leaching (or ages since deposition).

375

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

389 Specifically, mean concentrations of Cu, Zn and Pb exceed their respective probable effects  
390 levels at the three sites where APPs were most abundant. As a caveat, however, it must be  
391 borne in mind that these and other relevant standards implicitly assume that the metals are  
392 present in inorganic, bivalent form, while in APPs Cu is present in univalent form as Cu<sub>2</sub>O or Cu  
393 thiocyanate (Yebra et al., 2004) and other metals may be bound as specific biocidal,  
394 compounds (e.g. Zn pyrithione) whose toxicities are not well-defined.

395

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

405

406 Elevated but heterogeneous concentrations of Cu, Zn, Sn and Pb were also evident in the  
407 tissue of *H. diversicolor* sampled from locations impacted by contemporary boating activity or  
408 in the vicinity of abandoned boats; elevated Pb concentrations are, however, restricted to the  
409 latter locations, consistent with the application of Pb in historic antifouling formulations and in  
410 older non-antifouling paints (used on boat cabins and decks, for example; Booher, 1988).

411 Relative to tissue concentrations in the corresponding control samples, Cu exhibits  
412 considerably greater enrichment in animals from contaminated sites than Zn, despite broadly  
413 similar concentrations of these metals in contaminated sediments and in APPs. For example, at  
414 the University Marine Centre, mean Cu concentrations are six times higher than those at the  
415 control site whereas Zn concentrations are just 50 % greater. Accumulation of Cu in *H.*  
416 *diversicolor* has previously been linked to high environmental concentrations and there is  
417 evidence that the ragworm is unable to regulate Cu body concentrations in many locations,  
418 leading to bioaccumulation of the metal (Berthet et al., 2003). Conversely, Zn concentrations  
419 have been found to remain relatively constant in ragworms, regardless of external  
420 concentrations, with the animal appearing to regulate this metal to a certain extent, albeit by  
421 reducing energy reserves (Durou et al., 2005).

422

423 The widespread occurrence of Cu-rich APPs retrieved from the guts of individuals of *H.*  
424 *diversicolor* suggests that accumulation of the metal may, at least partly, take place through



425 the solubilisation of the metal in the digestive environment and transfer to the circulatory  
426 fluids. An *in vitro* study undertaken by Jones and Turner (2010) demonstrated that both  
427 surfactants and enzymes typical of the gut fluids of marine deposit-feeding organisms are able  
428 to facilitate the mobilisation of Cu from APPs into sea water through a series of complexation  
429 and exchange reactions. However, the measurements of the present study are unable to  
430 discriminate this mode of uptake from absorption of Cu via interstitial waters enriched in APP  
431 leachate.

432

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

448

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

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461

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