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3 **Spatial variation of trace metals within intertidal beds of native mussels (*Mytilus edulis*) and non-native**
4 **Pacific oysters (*Crassostrea gigas*): implications for the food web?**

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11
12 **Keywords:** Pacific oyster (*Crassostrea gigas*); Mussel (*Mytilus edulis*); Non-native species; Invasive species
13 Metals (Cd,Pb,Cu,Zn); North Sea; Trophic transfer; Multiple stressors

14
15 **Abstract**

16
17 Pollution is of increasing concern within coastal regions and the prevalence of invasive species is also rising. Yet
18 the impact of invasive species on the distribution and potential trophic transfer of metals has rarely been
19 examined. Within European intertidal areas, the non-native Pacific oyster (*Crassostrea gigas*) is becoming
20 established, forming reefs and displacing beds of the native blue mussel (*Mytilus edulis*). The main hypothesis
21 tested is that the spatial pattern of metal accumulation within intertidal habitats will change should the abundance
22 and distribution of *C.gigas* continue to increase. A comparative analysis of trace metal content (cadmium, lead,
23 copper and zinc) in both species was carried out at four shores in south-east England. Metal concentrations in
24 bivalve and sediment samples were determined after acid digestion by inductively coupled plasma—optical
25 emission spectrometry. Although results showed variation in the quantities of zinc, copper and lead (mg m^{-2}) in
26 the two bivalve species, differences in shell thickness are also likely to influence the feeding behaviour of
27 predators and intake of metals. The availability and potential for trophic transfer of metals within the coastal food
28 web, should Pacific oysters transform intertidal habitats, is discussed.

31

32

33 **Introduction**

34

35 The ecological impact of invasive species is a global issue that has resulted in significant displacement of native
36 fauna and economic impact (Rilov & Crookes, 2009). Increasing temperatures as a consequence of climate change
37 heightens the risk of biodiversity loss from non-native introductions, particularly in temperate and **P**polar
38 **R**regions (Sorte et al. 2010; de Rivera et al. 2011). Most studies of the impact of non-native species on
39 biodiversity focus on direct ecological effects, such as interspecific competition with native species (see Byers et
40 al. 2009 for review), predator prey interactions (Rilov, 2009), ecosystem engineering (Ruesink et al. 2005;
41 Crooks, 2009) and relative tolerance to environmental stress (Lenz et al. 2011). Amongst many anthropogenic
42 impacts that threaten biodiversity of the coastal zone, pollution can also be highly significant (Clark, 2001). The
43 impact of metals from mining operations, domestic discharges and as a component of anti-fouling paints on
44 individual species and species diversity has received widespread attention (Kennish, 1997; Kushel & Timperley
45 1999; Clark, 2001). Tolerance of non-indigenous species to metal-contaminated habitats may have facilitated
46 their spread (Piola & Johnston, 2009; McKenzie et al. 2012). Yet the impact of invasive species on the
47 distribution and potential trophic transfer of metals and other pollutants has rarely been examined. Buddo et al.
48 (2012) considered human health implications from harvesting the invasive Indo-Pacific green mussel *Perna*
49 *viridis* in the Caribbean and Hübner et al. (2010) investigated the potential release of cadmium from the erosion of
50 accumulated sediments within swards of the invasive cordgrass ~~grass~~ *Spartina anglica*. In freshwater systems the
51 invasive macrophyte *Eichhornia crassipes* (water hyacinth) is known to accumulate considerable amounts of
52 organic and metal contaminants (see Villamagna & Murphy (2010) for review).

53

54 Trace metals are naturally found within the environment, although levels may be elevated within industrial areas,
55 ports and marinas. For example copper is a constituent of antifouling paints and zinc is a prominent constituent of
56 anti-corrosion coatings and urban road run-off due to vehicle tyre wear (Councell et al.2004). Other metals,
57 including cadmium and lead, are used in a variety of industrial processes including colouring, plating and printing
58 (Guéguen et al. 2011). Some trace metals (e.g. copper and zinc) are considered essential for many invertebrates
59 and have a vital physiological role (Depledge & Rainbow, 1990), yet others (e.g. cadmium and lead) are non-

60 essential and need to be detoxified or excreted by organisms to avoid toxic effects (Phillips & Rainbow, 1989;
61 Depledge and Rainbow, 1990; Rainbow, 2002). Even essential metals can become toxic to organisms if uptake,
62 from either solution or in food, exceeds the combined rate of excretion and detoxification to reach and then rise
63 above levels that can be tolerated (Bryan, 1971; Rainbow, 2002). The uptake of trace metals by invertebrates may
64 occur directly across permeable membranes from the surrounding seawater, ingestion from water, suspended
65 organic matter and sediments, from food and also from metal-rich particles via pinocytosis (Depledge & Rainbow,
66 1990; Langston et al. 1998). Uptake may be influenced by a number of factors including temperature, salinity, pH
67 and the particular metal species (Depledge & Rainbow, 1990). For the oyster *Crassostrea gigas*, dissolved copper
68 in ambient water, as opposed to copper in phytoplankton food and sediments, has been shown to be the most
69 important source of copper in tissues (Ettajani et al. 1992).

70
71 Bivalves exhibit a range of behaviours and strategies that deal with the potential toxic effects of metals. Trace
72 metals can be excluded from ingestion and be removed in the pseudofaeces of oysters and mussels (Newell &
73 Jordan, 1983; Arifin & Bendell-Young, 1997). The regulation of metal concentrations as a detoxification strategy,
74 where whole body concentrations remain relatively constant and independent of [the](#) differences in bioavailability
75 at different sites, requires verification by experiments (Depledge & Rainbow, 1990; Rainbow & Dallinger, 1993).
76 However, partial regulation of zinc has been observed in the mussel *Mytilus edulis* (Lobel et al. 1982). Oysters, by
77 contrast are generally strong net accumulators of zinc (Rainbow, 1992; Wang & Rainbow, 2008; Giltrap et al.
78 2013). Accumulated metals may be divided in-to that which is metabolically available and a detoxified component
79 that is stored in tissues (Rainbow, 2007). The immobilisation and storage of accumulated and potentially toxic
80 metals is important in both oysters and mussels. However the physico-chemical form of storage can vary across
81 different tissues. For example metals can be stored as insoluble granules within lysosomes in the kidney
82 (*M.edulis*), and in the digestive gland and basal lamina in both oysters and mussels. Almeida et al. (1998) found
83 evidence of a possible regulatory mechanism in *C.gigas* for lead, whereby excess is directed to the shell through
84 the mantle. Some metals (e.g. Cu and Zn) can be compartmentalised and detoxified in the soluble phase by
85 binding to proteins known as metallothioneines (Phillips & Rainbow, 1989; Rainbow, 2002; Rainbow et al. 2007,
86 2011). These processes can render both Cu and Zn inactive within intracellular metabolic processes (Geffard et al.
87 2004; Rainbow, 2007). The accumulation of metals and other chemicals in molluscs are often measured as part of
88 widespread national and international monitoring programmes such as ‘Mussel Watch’ (e.g. Tripp, 1992). In
89 addition to the efficiency of detoxification mechanisms, the accumulation of metal in marine bivalves is
90 influenced by a range of other factors. These include the size, age and reproductive condition of the animals

91 (Langston et al. 1998; Bryan et al. 1985), uptake and efflux rates (Wang & Rainbow, 2008) and environmental
92 factors including salinity, the level of contamination and diet (Roesijadi & Robinson, 1994; Geffard et al. 2004;
93 Lekhi et al. 2008; Wang & Rainbow, 2008).

94
95 The trophic transfer of trace metals along food chains is common in aquatic environments (Wang, 2002; Rainbow,
96 2002, Rainbow et al. 2007, 2011; Rainbow & Smith, 2010; Guo et al. 2013). Yet transfer is controlled not only by
97 the concentrations of the metal but also by the food characteristics and physico-chemical form of the metal in the
98 prey, detoxification processes and the digestive and feeding physiology of the predator (Wang, 2002). Therefore
99 significant changes in the uptake of metals from organisms in the wild, and any consequential toxic effects via
100 trophic transfer, will depend on the physiology and tolerance levels of predatory species within the particular
101 ecosystem. Invertebrates from the same site will differ both in their total metal concentrations and partitioning of
102 metals in tissues (Rainbow et al. 2007, 2011; Guo et al. 2013). The trophic availability of metals in prey can be
103 dependent on the digestive and assimilative abilities of the specific predatory species (Rainbow & Smith, 2010).
104 Whelks and other gastropods can be important predators in intertidal benthic environments and the uptake of
105 metals from prey is the most important route of transfer (Blackmore & Wang, 2004; Guo et al. 2013).
106 Investigations of trophic transfer of metals between the rock oyster *Saccostrea cucullata* and whelk *Thais*
107 *clavigera* (Blackmore & Wang, 2004) showed not only that metals were transferred between [the](#) trophic levels but
108 [also](#) that cadmium, mercury and zinc had high potential for biomagnification in the food chain of rocky shores. In
109 laboratory experiments, over eight weeks there was a higher uptake of zinc, copper and cadmium by the
110 gastropod *Nassarius siquijorensis* feeding on the oyster *Crassostrea angulata* compared [with](#) other bivalves
111 (Guo et al. 2013).

112
113 The abundance and spatial extent of organisms that are accumulating metals and their importance to predators will
114 also determine what, if any, influence metals may have on the food web. Studies that describe broad-scale patterns
115 of bioaccumulation and availability in different organisms are required. This may be particularly important for
116 invading species that can often reach high densities.

117 Introductions of non-native molluscs have been relatively frequent and several species now form important
118 populations in coastal ecosystems (Eno et al. 1997; Miller et al. 2007). Pacific oysters are one of the most
119 'globalised' marine invertebrates, having been introduced for aquaculture and cultivation into 66 countries outside
120 [of](#) their native range (Ruesink et al. 2005). In several temperate regions, rising sea temperature has resulted in
121 successful spawning and subsequent wild settlement beyond cultivated areas (Reise 1998; Dutertre et al. 2010;

122 Ruesink et al. 2005, Ruesink 2007). Pacific oysters will settle preferentially on conspecifics and in parts of
123 Europe, intertidal reefs consisting of a dense concretion of oysters have now developed on previously muddy and
124 rocky habitats (Reise, 1998; Lejart & Hily, 2011). In the Wadden Sea, Pacific oyster reefs now occupy large areas
125 of former beds of the native mussel (*Mytilus edulis*) (Diederich, 2005; Nehls & Buttger, 2007) and there are now
126 very few mussel beds without *C.gigas*. In the past decade, the abundance of wild *C.gigas* has increased on the
127 south coast of England and smaller reefs and dense aggregations are occurring locally on a range of substrata
128 including mussel beds (Herbert et al. 2012).

129
130 Due to efficient detoxification mechanisms and high uptake rate (Rainbow, 1992; Roesijadi, 1996) cultivated
131 oysters (*Crassostrea* spp.) are known to accumulate particularly high concentrations of some metal elements such
132 as zinc (Rainbow, 1992; Wang & Rainbow, 2008; Giltrap et al. 2013). It is reasonable therefore to hypothesise
133 that non-native wild *C.gigas* will also similarly accumulate metals and that the magnitude of accumulation may
134 differ from field measurements in native bivalves. Should *C.gigas* displace native mussel beds then the spatial
135 distribution and availability of metals across intertidal habitats may change. Intertidal mussels can be important
136 prey for invertebrates including shore crabs (*Carcinus maenas*) (Dare et al. 1983; Frandsen & Dolmer 2002), dog
137 whelks (*Nucella lapillus*) (Crothers, 1985), starfish (*Asterias rubens*) (Dolmer, 1998) and shorebirds including
138 Oystercatcher (*Haematopus ostralegus*) (Goss-Custard, 1996; Scheiffarth et al. 2007), Herring gull (*Larus*
139 *argentatus*) and Eider duck (*Somateria mollissima*) (Scheiffarth et al. 2007). In North America, crabs, whelks and
140 sunstars are known predators of *C.gigas* at aquaculture sites (Quayle, 1964). In laboratory experiments, Dare et al.
141 (1983) observed predation of *C.gigas* of up to 55–60 mm by large shore crabs (*C. maenas*), yet stated that most
142 crab predation at aquaculture sites is likely to be on smaller oysters (40–45 mm). Little is known about bird
143 predation on wild Pacific oysters, however Oystercatchers have been observed taking *C.gigas* at shell lengths
144 between 17–68 mm, though the size range was dependent on season (Markert et al. 2013). Herring gulls are also
145 known to feed on wild Pacific oysters (Scheiffarth et al. 2007; Markert et al. 2013).

146 The main hypothesis tested therefore is that the spatial pattern of metal accumulation in intertidal habitat will
147 change should wild *C.gigas* increase and displace native fauna. The main objectives are (i) to measure the
148 concentration of metals (Cd, Pb, Cu, Zn) within *M.edulis* and *C.gigas* populations on rocky shores where the two
149 species currently coexist and (ii) to predict changes in their spatial distribution should *M.edulis* be displaced by

150 *C.gigas*. Implications of displacement of *M.edulis* by *C.gigas* for the potential trophic transfer of metals in the
151 coastal food web are discussed.

152 **Materials and Methods**

153

154 *Study area and sampling sites*

155 In Kent, south-east England, four shores on the open coast were selected where Pacific oysters have colonised
156 over the past decade (Fig. 1). Epple Bay, Nayland Rock and Foreness Point consist of chalk bedrock with adjacent
157 sand and muddy sediments, whereas Longrock is a mixed sediment shore with flint and shell debris. The mouth of
158 the Thames Estuary that flows through the port of London is about 30 km west of Longrock, yet most of the local
159 coastal land use is suburban residential housing and tourist development. A Pacific oyster farm and hatchery is
160 located 9 km east of Whitstable and the nearest port is at Ramsgate, where a dredge spoil dumping site is located
161 offshore. Salinity is 35 psu and mean sea temperature range is 5–18 °C. Tidal range is 5 m and residual currents
162 run west to east from the Thames estuary along the north Kent coast and north-east from Ramsgate. The upper
163 part of each shore is mostly colonised by fucoid algae, yet mussel beds (*M. edulis*) dominate large patches of the
164 middle and lower shore. Pacific oysters can be found across the whole intertidal zone, especially on the middle
165 and lower shore amongst the mussel beds. Peak densities of live oysters recorded at the four sampling sites (Fig.1)
166 during baseline surveys between July 2007 and July 2008 (McKnight, 2012) were Longrock (6 ind. m⁻²), Epple
167 Bay (181 ind. m⁻²), Naylands Rock (44 ind. m⁻²) and Foreness Point (32 ind. m⁻²). At Epple Bay, densities were
168 sufficiently high to form reef-like aggregations of contiguous oysters. In many of these aggregations, oysters were
169 vertically orientated and some individuals attain shell lengths >10 cm. The sampling sites to the east of Longrock
170 are within part of the North East Kent European Marine Site (NEKEMS), which has been designated as Special
171 Area of Conservation (EU Habitats Directive) and Special Protection Area (EU Birds Directive).

172

173 *Field sampling*

174

175 Samples of Pacific oysters, mussels and sediments were collected in April 2012. Thirty adult *C.gigas* and *M.edulis*
176 were collected at Mean Low Water from each shore, with fifteen individuals taken in two patches at least 100 m

177 | apart. Mussel length was between 25–35 mm, which from population size–frequency histograms would indicate
178 | they were at least one year of age (Wright & Bailey, 2009). Oysters sampled were from the most frequent size
179 | classes (50–70 mm) which from population size–frequency histograms (McKnight & Herbert, unpublished data)
180 | are also estimated to have recruited at least one to two years previously. Samples were frozen at -20°C in labelled,
181 | sealed plastic bags. Data on the size, density and biomass of two intertidal mussel beds sampled in 2008 at Shell
182 | Ness and Pegwell (Fig 1) were obtained from Wright & Bailey (2009). These estimates of mean biomass and
183 | density were based on monthly grab-samples (n =5) of 0.1 m². For metal analysis, three surface sediment
184 | samples (upper 5 cm) of ~250 g were taken at each site within 1 m of the shellfish samples, placed in a labelled
185 | bag and frozen. These samples together with a field assessment of the proportion of gravel, sand, silt and clay at
186 | each sampling site were used to classify beach sediments according to Folk (1954).

187

188 | *Laboratory analysis*

189

190 | Defrosted shellfish were opened with a knife to remove the flesh. Oyster and mussel tissues were removed from
191 | shells and dried at 60–80°C until constant and mean dry weight was calculated. The dried shellfish were weighed
192 | and homogenised separately using a mortar and pestle. Sub-samples of invertebrate material (0.200 g +/- 0.001)
193 | were weighed into digestion vessels and 1 ml 37% HCl and 4 ml 70% HNO₃ were added. After microwave
194 | digestion (Anton Paar Multiwave 3000; 5 min at 750 W, then 25 min at 100 W; Maichin et al. 2000) samples were
195 | filtered through Whatman No. 42 filter papers before being made up to a final volume of 50 ml using deionised
196 | distilled water.

197 | Sub-samples of sieved sediments (0.300 g +/- 0.001 of particle size < 212 µm) were then weighed into digestion
198 | vessels and 6 ml of *aqua regia* was added (Millward & Kluckner, 1989). *Aqua regia* is a widely used and
199 | accepted extract for the determination of the pseudo-total metal concentration in sediments and weaker extractants
200 | may fail to dissolve all metals from anthropogenic sources (Peña-Icart et al., 2011).

201

202 | After microwave digestion (20 minutes at 200°C) samples were again filtered through Whatman No. 42 filter
203 | papers before being made up to a final volume of 50 ml with deionised distilled water. All equipment for digestion

204 and filtering was acid conditioned in 10 % HNO₃. Fisher Primar Plus trace metal grade reagents were used
205 throughout. Metal concentrations in digested samples were determined by inductively coupled plasma--optical
206 emission spectrometry (ICP-OES) for metal analysis (Varian Vista-Pro in axial configuration). Analytical validity
207 was ensured by the digestion and analysis of certified reference materials (NWRI TH-2 harbour sediment) and
208 process blanks. Recoveries were between 70--80% and precision (%RSD) was between 2.6--3.8 (Table S1).

209

210 *Statistical analysis*

211

212 The significance of the differences between the main effects of Species and Site were tested using Two-Way
213 ANOVA in 'R' (R Core Team, 2013). Sediment concentrations were not included in the analysis as the principal
214 aim of the investigation concerns the differences in concentrations between the two shellfish species. One-Way
215 ANOVA was used to test for site-specific differences between each combination of metal and species. In all cases
216 the White correction for heteroscedasticity has been used (White, 1980). Multiple comparisons use the sandwich
217 estimators for the covariance matrix. Multiple comparisons (after allowing for unbalanced samples and
218 heteroscedasticity) were adjusted using Tukey's HSD. This allowed robust pairwise comparisons to be made
219 between mean log concentrations of each metal across sites.

220

221 **Results**

222 Using the Folk Classification (Folk, 1954), both Longrock and Epple Bay have sediments consisting
223 predominantly of muds (Longrock, *Gravel-sandy Mud* ((g) sM); Epple Bay, *Muddy-sand* (mS)) whereas Nayland
224 Rock and Foreness Point were classified as *slightly gravelly muddy Sand* ((g) mS). Compared with some other
225 European regions (Reise 1998; Lejart & Hily, 2011), densities of Pacific oysters on most shores along the Kent
226 coast are relatively low (McKnight, 2012). The mean metal concentrations (Cd, Pb, Cu, Zn) in sediments, mussels
227 and oysters ($\mu\text{g g}^{-1}$ dry weight) from each shore are presented in Table 1. Observed concentrations of metals in
228 sediments are well below thresholds in all commonly used sediment quality values, as summarised by Hübner et
229 al. (2009). As shown in the boxplots of Figure S1, the range of values of metal concentrations for individual
230 mussels and oysters can be high, particularly lead and cadmium at Epple Bay and Nayland Rock.

231

232 Figure 2 shows the Mean \pm 95% Confidence Intervals of metal concentrations ($\mu\text{g g}^{-1}$ dry weight) within sediment
233 and shellfish at sampling sites on the north coast of Kent, UK, in April 2012. Cadmium (Cd), Lead (Pb), Copper
234 (Cu), and Zinc (Zn). Bootstrapped 95% confidence intervals were calculated for all cell means using 1000 random
235 draws with replacement using the procedure in the Hmisc package in R (Harrel, 2014). Significant differences ($p \leq$
236 0.05) are apparent for most comparisons between mussels and oysters.

237

238 Two-Way ANOVA tables are presented in Table S2. The analysis confirmed the significance of the pattern of
239 differences between means shown by Figure 2. Levene's test showed significant heteroscedasticity in the residuals
240 for all metals. Therefore White-corrected covariance matrices were included in all models in order to adjust for
241 the lack of homogeneity. The reported p-values for the main effects of the two-way ANOVAs were based on
242 Type 3 sum of squares for each metal. Sediment was not included in the test which looked at differences between
243 the two species of shellfish. The analysis indicates highly significant effects of species on the concentration of all
244 metals after holding for site effects and site:species interactions. The significance of main effects is questionable
245 if interactions are apparent which change the sign of the response. However, inspection of Figure 2 shows clear
246 differentiation of means for the species effect calculated using non-parametric bootstrapping and consistency in
247 the pattern of difference. The significance of the main effect for species is therefore reliable.

248

249 There was no west-east gradient in the concentration of metals within the sediments sampled along the coast,
250 although mean levels of lead, copper and zinc at the two western sites (Longrock and Epple Bay) were
251 approximately double than those measured at the eastern sites (Nayland Rock and Foreness). There was no
252 relationship between concentrations of metals in sediments and those in the shellfish of any species, although this
253 was not statistically tested due to few sampling sites.

254

255 *Cadmium*

256 Levels of cadmium did not significantly differ in sediments at the four sites (Table S1). Yet levels were
257 significantly greater in shellfish, except for at Longrock where the difference in levels between sediments and

258 | mussels was not statistically significant. There was no significant difference in the levels in Pacific oysters
259 | between sampling sites however concentrations were significantly higher than both sediments and mussels at each
260 | site. Mean levels in Pacific oysters ($2.2 \mu\text{g g}^{-1}$ dry weight), were over three times that in mussels ($0.7 \mu\text{g g}^{-1}$ dry
261 | weight) and at each site were always significantly higher than mussels. Levels in Longrock mussels were
262 | significantly less than at other sampling sites.

263

264 *Lead*

265 | Levels of lead in sediments at the two western sites were almost double of those measured at the two eastern sites,
266 | with mean levels at Epple Bay the highest ($10.9 \mu\text{g g}^{-1}$ dry weight). In shellfish, mean levels at each site were
267 | always less than in the sediments, although at Longrock and Naylands Rock the difference between mussels and
268 | sediments was not significant. In contrast to the other metals examined, levels in oysters were always significantly
269 | less than in mussels.

270

271 *Copper*

272 | In sediments, levels at the two western sites were more than double those of the two eastern sites.

273 | At all sites, levels in Pacific oysters were significantly higher than both mussels and sediments; mean levels in
274 | Pacific oysters were over forty-~~five~~ times greater than in mussels. Pacific oysters collected from Foreness had
275 | highest levels of any site (mean = $516 \mu\text{g g}^{-1}$ dry weight) and were significantly greater than the samples from
276 | Epple Bay and Nayland Rock.

277

278 *Zinc*

279 | Significantly higher concentrations occurred in Pacific oysters compared with~~to~~ both mussels and sediments.
280 | Across the study area, mean zinc concentrations in Pacific oysters were almost thirty times greater than in
281 | mussels, with highest mean levels measured at Foreness, ($2600 \mu\text{g g}^{-1}$ dry weight) in the east of the study area. In
282 | mussels, levels were significantly less at Longrock compared with~~to~~ the other sites and there was no significant
283 | difference between levels in mussels and sediments at this location.

284

285 | Mean flesh dry weights of sampled mussels and Pacific oysters were 0.18 and 0.62 g, respectively. Local mussel
286 | beds are of mean density 2475 ind. m⁻² (Wright & Bailey, 2009) and Pacific oysters reef ~ 200 ind. m⁻²
287 | (McKnight, 2012). Estimated mean metal concentrations (mg dry weight m⁻²) within both mussel beds and Pacific
288 | oyster reefs are presented in Table 2. Although the mean bivalve density and biomass of a mussel bed is
289 | considerably higher than that of a Pacific oyster reef, the average accumulation of copper and zinc (µg g⁻¹ dry
290 | weight) is much greater in *C.gigas* compared ~~with~~ mussels. Yet lead (µg g⁻¹ dry weight) is higher in mussels than
291 | oysters. Quantities of these metals within intertidal habitats are therefore projected to change should *C.gigas* reefs
292 | form and displace the mussel beds. Zinc concentrations (mg m⁻²) are predicted to be approximately eight times
293 | greater within a Pacific oyster reef compared ~~with~~ a mussel bed and copper (mg m⁻²) is estimated to be thirteen
294 | times higher. Yet quantities of lead (mg m⁻²) could be over twenty times less in a Pacific oyster reef compared
295 | ~~with~~ a mussel bed, although cadmium levels are likely to be unchanged.

296

297 | **Discussion**

298

299 | Observations of relative metal accumulation in oysters and mussels support data obtained elsewhere, albeit mostly
300 | from cultivated shellfish (Table 3). Mussels accumulated higher quantities of lead whereas oysters accumulated
301 | greater quantities of copper and zinc. The range of metal concentrations obtained for individual bivalves was high,
302 | particularly at Epple Bay and Nayland Rock, however this is typically observed at other locations (Bryan et
303 | al.1985).- The study area on the north Kent coast is relatively uncontaminated; although levels of zinc, lead and
304 | copper were greater in sediments at the two western sites this is most likely because metals were bound to the
305 | finer clays and muds sampled at these locations. Much higher levels of zinc, copper and cadmium have been
306 | accumulated in Pacific oysters at more contaminated locations (Table 3; Amiard et al. 2008). Although there have
307 | been other recent determinations of metals in mussels from this region of ~~S~~outh-~~E~~ast England (BODC, 2014),
308 | there are no comparative values for wild Pacific oysters available. The levels of each of the four metals found in
309 | mussels are comparable with data obtained from shellfish cultivation regions in the UK over the past 20 years
310 | (Table 3). Values of lead and cadmium in Pacific oysters are also similar to other determinations, though levels of
311 | copper and zinc are higher than previous mean values for England and Wales and the loughs of Northern Ireland

312 (Table 3) (although mean values of zinc in Carlingford Lough obtained between 2010–2012 was 1702 $\mu\text{g g}^{-1}$ dry
313 wt. (BODC, 2014).

314

315 As the purpose of the investigation was to determine the magnitude of potential metal transfer should the bivalves
316 be consumed by predators within the ecosystem, shellfish were not purged of faeces prior to analysis. Values
317 obtained in this study may therefore be slightly higher than other analyses where bivalves have been purged or
318 undergone depuration. However from estimates of annual biodeposition rates in *C.gigas* (Nishikawa-Kijomura,
319 1978) quantities of Cu and Zn present in the gut is likely to be less than 1% of flesh estimates. Standardisation of
320 metal concentrations to allow for variation in reproductive condition between the two species was not carried out.

321 For mussels, spawning is known to occur during April–May in this region (Wright & Bailey, 2009) and Pacific
322 oysters in summer, assuming sustained temperatures for gametogenesis (Miossec et al. 2009). Bryan et al. (1985)
323 reviewed the influence of sampling period for both species and recommended metals analysis of mussels in late
324 winter to avoid variations in concentration due to food supply and gonad development. In *C.gigas*, lower metal
325 concentrations in winter and summer are attributable to reduced dry mass and dilution due to gonad development,
326 respectively. In field populations of *C.gigas* in the Bay of Bourgneuf (France) concentrations of Cd, Cu, Zn, Cd
327 and Pb decreased in the late spring and early summer concomitantly with the increase of soft tissue weight and
328 sexual maturity. Inter-annual variations in concentrations of the metals were also associated with weight changes
329 (Amiard & Berthet, 1996). Therefore, taking account of possible variation in the two species in respect of
330 differing reproductive periods our estimates of metal concentrations are likely to be below annual maximum levels
331 for mussels, whereas estimates in oysters are probably closer to the annual maximum. In the Bay of Bourgneuf,
332 Pb and Cd concentrations in oysters were not correlated with the age or weight; however, Cu and Zn
333 concentrations generally did increase with age (Amiard & Berthet, 1996). Concentrations of Cd and Zn measured
334 in sediments and in *M.edulis* (four size classes, 35 to >50 mm) from two estuaries in South-east England
335 decreased between December and August in both the years studied (Wright & Mason, 1999). However, overall
336 differences in mean concentrations of Cd and Zn were much greater than variation attributed to shellfish age and
337 sampling season.

338 In this study, the considerably high species-specific differences in the concentration of these particular metals are
339 likely to be significantly greater than [the](#) variations associated with season and size of shellfish and are consistent
340 with the overall pattern of accumulation of these metals in the two species (Table 3).

341
342 Troost et al. (2009) argue that food intake of wild Pacific oysters may be greater than mussels due to the oyster's
343 high filtration rate and the larger roughness and height of oyster beds, which create more near-bed turbulence and
344 facilitates efficient ingestion. The quantities of zooplankton ingested may also be determined by near-bed
345 roughness differences (Troost et al. 2009). Although untested, turbulence may be different in wild reefs than
346 where *C.gigas* is cultivated in bags on trestles, which may cause differences in accumulated metals due to varied
347 efficiency and rate of ingestion. Variation in the quantities of accumulated metals within the two species is likely
348 to be the result of different ingestion efficiencies, food type and the ability to regulate different metals. Compared
349 [with](#) oysters, zinc appears to be regulated more efficiently in mussels (Lobel et al. 1982). The ability of *M.edulis*
350 to limit the accumulation of copper is dependent on which tissues and organs are examined, [the](#) exposure period
351 and [the](#) levels of contamination (Amiard-Triquet et al.1986). In this study, levels of copper were high in both
352 species and particularly oysters. However the metal regulatory abilities of each species need to be verified.

353
354 The trophic transfer of trace metals along food chains is common in aquatic environments (Rainbow, 2002,
355 Rainbow et al. 2007, 2011; Wang, 2002; Guo et al. 2013). Should *C.gigas* increase and replace beds of native
356 *M.edulis*, what effect might this have on the trophic transfer of metals in coastal habitats, and what, if any, are the
357 ecological implications? This will depend on whether potential predators can access and assimilate metals within
358 the oysters, the physico-chemical form of the metal in the oysters (Rainbow et al. 2002, 2011), detoxification
359 processes and the digestive and feeding physiology of the predator (Wang, 2002). Although experiments have
360 shown that *C.gigas* of up to 55-60 mm shell length can be consumed by shore crabs (*Carcinus maenas*) (Dare et
361 al. 1983), significant predation by invertebrates of adult *C.gigas* in the North Sea has not been observed (Troost,
362 2010), presumably due to [the](#) thickness of shells. Oystercatchers (*Haematopus ostralegus*) may prise-off and
363 consume smaller Pacific oysters (Markert et al. 2013), however their abundance on former mussel beds in the
364 Wadden Sea has significantly declined since colonisation by *C.gigas* (Scheiffarth et al. 2007).

365

366 Little predation was observed during experiments on *C.gigas* interactions on rocky shores (Ruesink, 2007).
367 Therefore, although Pacific oysters can accumulate larger quantities of copper and zinc than mussels, because
368 adult oysters are relatively inaccessible to predators, the displacement of mussel beds and other intertidal habitats
369 is unlikely to increase the trophic transfer of these metals to wild predators within the ecosystem. On the contrary,
370 the transfer of lead to predators, such as the gastropod *Nucella lapillus* (Crothers, 1985), may actually reduce if
371 mussels cannot be exploited and predators switch to alternative food sources.

372

373 Spatial and temporal variation in abundance and the extent to which mussels and oysters are significant prey is
374 important when considering trace metal availability to the coastal food web. Inter-annual recruitment of mussels is
375 known to vary considerably (Dare et al. 2004) and therefore the size and density of intertidal beds will also
376 change. The size-frequency of the mussel population varies throughout the year and it is known that
377 concentrations of metals can fall with increasing weight (Phillips, 1980). Although dense aggregations and small
378 reefs of Pacific oysters are now occurring in southern England, the extent to which *C.gigas* will persist, cause
379 extensive reef formation and thus change the intertidal habitat will depend on the magnitude and frequency of
380 recruitment and mortality. There is strong evidence to suggest that gametogenesis, spawning, larval development
381 and survival are determined by the attainment of threshold temperatures (Miossec et al. 2009) which have
382 increased in frequency within the past two decades (Dutertre et al. 2010). Recently, native species of rocky shores
383 at the northern edge of their biogeographical range have spread in response to warming (Hawkins et al. 2009), so
384 it is likely that *C.gigas* will also spread from established strongholds (Pinnegar et al. 2012). Unlike mussels,
385 *C.gigas* can potentially occupy all levels of the shore and have been found at Mean High Water on sea walls and
386 across other habitats including intertidal beds of the polychaetes *Sabellaria spinulosa* and *Lanice conchilega*
387 (McKnight 2012). The potential change in metal distribution across the shore could therefore be much greater
388 should the abundance of Pacific oysters increase as predicted. Although observed in the Wadden Sea (Smaal et al.
389 2005), there is little evidence of widespread settlement of *C.gigas* below the intertidal zone in the UK and
390 therefore subtidal beds of *M.edulis* are likely to be unaffected. Subtidal *M.edulis* may resupply affected intertidal
391 areas and enable survival of mussels, at least in patches. In the Dutch Wadden Sea, some recovery of mussel beds
392 has been observed (Nehls & Büttger 2007) and mussels have been found to colonise the interspaces between
393 oyster shells, so some intertidal co-existence appears likely even if *C.gigas* becomes dominant (Diederich, 2005;
394 Eschweiler & Christensen 2011).

395

396 Although untested, it is possible that elevated levels of particular metals will occur in Pacific oyster biodeposits,
397 including faeces and pseudofaeces within the sediment surrounding the oysters, as shown under experimental
398 conditions (Nishikawa-Kjnomura, 1977). If this is evident in the wild, constituent metals could be passed along
399 food chains as the invertebrate diversity amongst the shell interspaces and in sediments below the reefs of *C.gigas*
400 can be higher than the surrounding sediments (Lejart & Hily, 2011). Potentially at least, prey items within
401 biodeposits amongst the oyster interspaces might be exploited by foraging avian predators (Escapa et al. 2004).
402 The possibility for changes in trophic transfer of metals may therefore extend beyond the individual Pacific
403 oysters themselves and warrants further investigation. Bryan & Langston (1992) specifically highlighted the need
404 for more studies on the impacts of elevated levels of inorganic and organometals on coastal birds.

405

406 Compared ~~with~~ other regions of the world, the north coast of Kent is relatively uncontaminated and wild
407 harvesting of Pacific oysters for human consumption is not currently widespread, though is increasing in areas
408 where they are forming dense aggregations. Maximum permitted levels of metals within food for human
409 consumption can vary between countries (Amiard et al. 2008; Guéguen et al. 2011), however maximum
410 concentrations of the bioavailable component of both essential and non-essential metals in *C.gigas* have been
411 recorded above food safety limits in contaminated regions (Bragigand et al. 2004; Amiard et al. 2008; Osuna-
412 Martínez et al. 2011; He & Wang, 2013).

413

414 **Conclusion**

415

416 The main hypothesis tested, that the spatial pattern of metal accumulation within intertidal habitats will change
417 should the abundance and distribution of wild *C.gigas* continue to increase and displace *M.edulis*, is supported by
418 our analysis. Quantities of zinc and copper and lead (mg m^{-2}) potentially available for consumption by predators
419 are likely to change significantly. Yet, notwithstanding differences in the bioaccumulation of metals in the two
420 bivalves and their potential availability, any broad-scale changes in trophic transfer of metals will also be
421 dependent on whether predators are able to co-exist with and exploit the new invader for food. If the invading

422 | species and native species are able to co-exist then any change in trophic transfer is likely to be affected by the
423 | selective feeding behaviour of the predators and the relative changes in density of interacting species.

424 | There remains significant uncertainty about the ecological effects of metals on aquatic assemblages (Langston et
425 | al, 1998; Mayer-Pinto et al. 2010). The potential for significant spatial variation in trophic transfer of metals as a
426 | result of invasions of non-native species certainly exists. Whether this has any significant impact ecologically or
427 | on human health is also likely to be dependent on local levels of contamination.

428

429

430

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435

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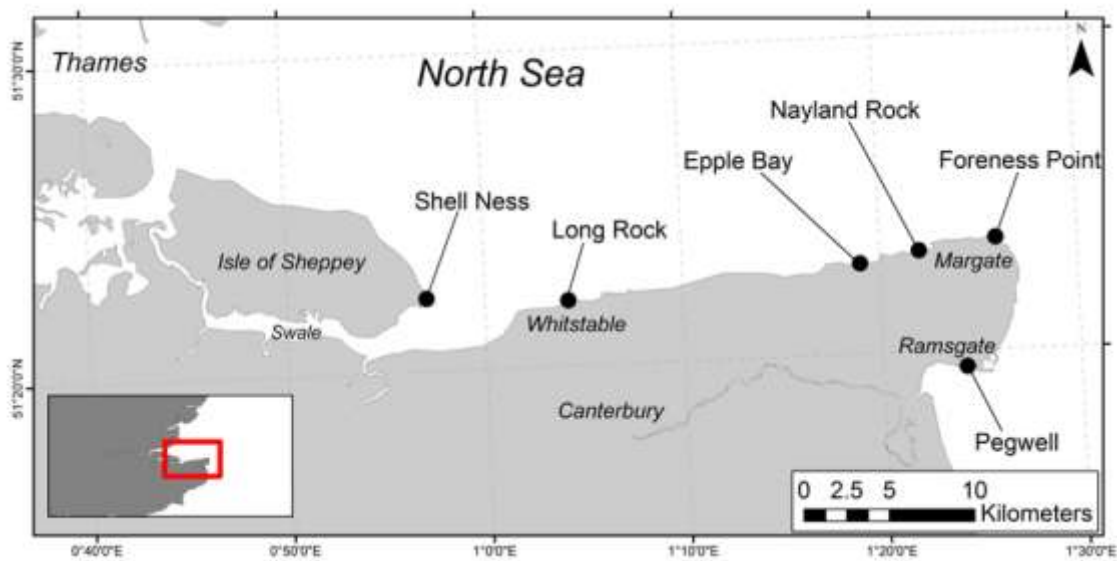
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691 **Fig 1.** The Thanet coast located in Kent (south-east England), showing mussel and oyster sampling sites at Long
692 Rock, Epple Bay, Nayland Rock and Foreness Point. Estimates of mussel density and biomass were obtained from
693 Shell Ness and Pegwell (Wright & Bailey, 2009). The main port is at Ramsgate.

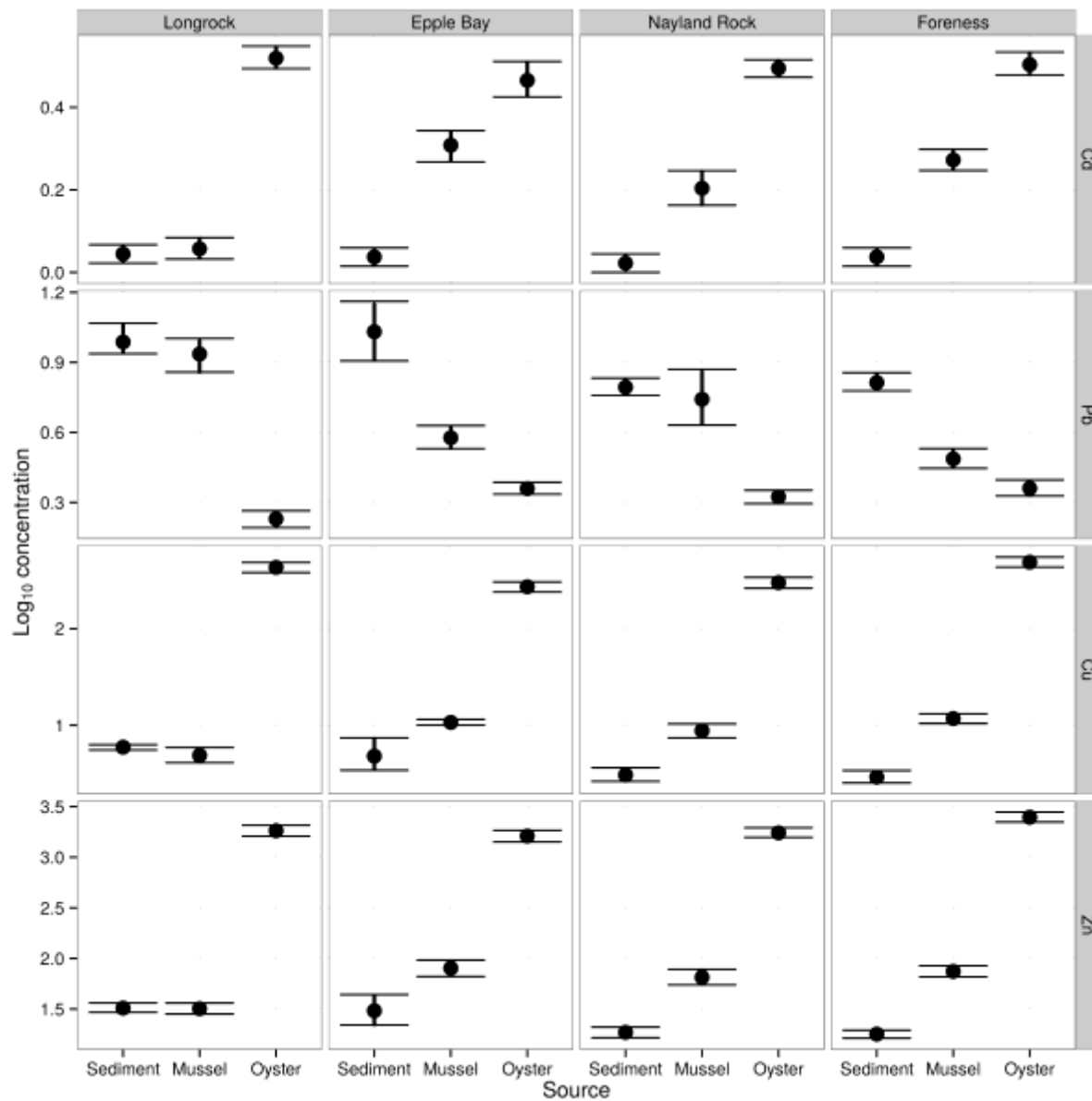
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700 **Fig 2.** Mean \pm 95% Confidence Intervals of metal concentrations ($\mu\text{g g}^{-1}$ dry weight) within sediment and shellfish
701 at sampling sites along the north coast of Kent, UK, in April 2012. -Cadmium (Cd); Copper (Cu); Lead (Pb) and
702 Zinc (Zn). Significant differences are apparent for most comparisons between the shellfish species.

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708 **Table 1:** Mean metal concentrations ($\mu\text{g g}^{-1}$ dry weight) and $\pm\text{SD}$ (italics) in each source from each shore

	Sediments					<i>M.edulis</i>					<i>C.gigas</i>				
	Longrock	Epple Bay	Naylands Rock	Foreness	Mean (SD)	Longrock	Epple Bay	Naylands Rock	Foreness	Mean (SD)	Longrock	Epple Bay	Naylands Rock	Foreness	Mean (SD)
Cd	0.1 (0.08)	0.1 (0.09)	0.1 (0.08)	0.1 (0.09)	0.09 (0.09)	0.2 (0.22)	1.1 (0.49)	0.7 (0.44)	0.9 (0.33)	0.7 (0.37)	2.4 (0.62)	2.1 (1.07)	2.1 (0.63)	2.2 (0.61)	2.19 (0.73)
Pb	9.1 (3.46)	10.9 (5.51)	5.3 (0.89)	5.6 (0.98)	7.7 (2.71)	8.3 (2.91)	3.0 (1.45)	6.9 (7.86)	2.2 (1.06)	5.1 (3.32)	0.7 (0.41)	1.3 (0.38)	1.1 (0.41)	1.4 (0.58)	1.14 (0.44)
Cu	5.00 (0.61)	4.80 (3.97)	2.2 (0.92)	2.0 (0.76)	3.48 (1.56)	4.50 (2.77)	9.9 (2.23)	8.7 (4.10)	11.30 (3.47)	8.59 (3.14)	453.80 (158.15)	285.70 (88.16)	309.30 (138.79)	515.70 (187.83)	391.36 (143.23)
Zn	31.80 (6.61)	34.5 (20.66)	17.9 (3.87)	17.1 (2.72)	25.32 (8.46)	35.0 (21.71)	91.30 (52.29)	71.9 (32.10)	77.9 (26.30)	69.06 (33.10)	1912.80 (544.07)	1712.1 (533.20)	1663.7 (535.53)	2600.0 (855.35)	1972.17 (617.04)

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716 **Table 2.** A comparison of estimated mean metal concentrations (mg dry weight m⁻²) obtained in this study within
717 an intertidal mussel bed (mussel density 2475 ind. m⁻², Wright & Bailey, 2009) and Pacific oysters reef (oyster
718 density ~ 200 ind. m⁻² (McKnight, 2012). The mean metal concentrations (Cd, Pb, Cu, Zn) in oysters and mussels
719 (µg g⁻¹ dry weight) from each shore are presented in Table 1. See text for further details.

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	Cadmium (mg dry weight m ⁻²)	Lead (mg dry weight m ⁻²)	Copper (mg dry weight m ⁻²)	Zinc (mg dry weight m ⁻²)
Mussels (<i>M.edulis</i>)	0.3	2.3	3.8	30.8
Wild Pacific oysters (<i>C.gigas</i>)	0.3	0.1	48.5	244.5

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722 **Table 3.** Mean metal concentration ($\mu\text{g g}^{-1}$ dry weight) in *M. edulis* and *C. gigas* from UK and France. Samples of *C. gigas* used in this study were from wild populations.

723 Contaminated samples from Ronce les Bains and the Gironde Estuary were obtained in 1990s (Bragigand et al. 2004, Table 1).

Location	Mussels (<i>Mytilus edulis</i>)				Pacific oysters (<i>Crassostrea gigas</i>)				References
	Cadmium $\mu\text{g g}^{-1}$	Lead $\mu\text{g g}^{-1}$	Copper $\mu\text{g g}^{-1}$	Zinc $\mu\text{g g}^{-1}$	Cadmium $\mu\text{g g}^{-1}$	Lead $\mu\text{g g}^{-1}$	Copper $\mu\text{g g}^{-1}$	Zinc $\mu\text{g g}^{-1}$	
North Sea, Kent.	0.7	5.1	8.6	69	2.2	1.1	391	1972	This study
Irish Sea (Mean 2008– 2012). ^a	1.3	1.9	6.7	91	1.2	0.9	91	1196	BODC, 2014.
England & Wales (Mean 1995–1996). ^a	1.3	5.5	9.0	110	1.05	1.0	208	1000	Jones & Franklin, 2000.
Atlantic, Arcachon Bay, France	-	-	-	-	2.0	-	100	2237	Sources in Table 1 of Bragigand et al. 2004.
Atlantic, Pen Be, France	-	-	-	-	0.7	-	73	1418	Sources in Table 1 of Bragigand et al. 2004.
Atlantic, Bourgneuf Bay, France	-	-	-	-	2.3	-	210	2209	Sources in Table 1 of Bragigand et al. 2004.
Atlantic, Ronce les Bain, France	-	-	-	-	6.7	-	220	2782	Sources in Table 1 of Bragigand et al. 2004.
Atlantic, Gironde Estuary, France	-	-	-	-	75	-	1041	4964	Sources in Table 1 of Bragigand et al. 2004.

724 ^a wet weight data multiplied by 5 to convert to dry weight (OSPAR, 2009; Amiard et al. 2008)

