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

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Abstract

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

Introduction

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

Trace metals are naturally found within the environment, although levels may be elevated within industrial areas, ports and marinas. For example copper is a constituent of antifouling paints and zinc is a prominent constituent of anti-corrosion coatings and urban road run-off due to vehicle tyre wear (Councell et al.2004). Other metals, including cadmium and lead, are used in a variety of industrial processes including colouring, plating and printing~~,~~ (Guéguen et al. 2011). Some trace metals (e.g. copper and zinc) are considered essential for many invertebrates and have a vital physiological role (Depledge & Rainbow, 1990), yet others (e.g. cadmium and lead) are non-essential and need to be detoxified or excreted by organisms to avoid toxic effects (Phillips & Rainbow, 1989; Depledge and Rainbow, 1990; Rainbow, 2002). Even essential metals can become toxic to organisms if uptake, from either solution or in food, exceeds the combined rate of excretion and detoxification to reach and then rise above levels that can be tolerated (Bryan, 1971; Rainbow, 2002). The uptake of trace metals by invertebrates may occur directly across permeable membranes from the surrounding seawater, ingestion from water, suspended organic matter and sediments, from food and also from metal-rich particles via pinocytosis (Depledge & Rainbow, 1990; Langston et al. 1998). Uptake may be influenced by a number of factors including temperature, salinity, pH and the particular metal species (Depledge & Rainbow, 1990). For the oyster *Crassostrea gigas*, dissolved copper in ambient water, as opposed to copper in phytoplankton food and sediments, has been shown to be the most important source of copper in tissues (Ettajani et al. 1992).

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

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

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

The abundance and spatial extent of organisms that are accumulating metals and their importance to predators will also determine what, if any, influence metals may have on the food web. Studies that describe broad-scale patterns of bioaccumulation and availability in different organisms are required. This may be particularly important for invading species that can often reach high densities.
Introductions of non-native molluscs have been relatively frequent and several species now form important populations in coastal ecosystems (Eno et al. 1997; Miller et al. 2007). Pacific oysters are one of the most 'globalised' marine invertebrates, having been introduced for aquaculture and cultivation into 66 countries outside ~~of~~ their native range (Ruesink et al. 2005). In several temperate regions, rising sea temperature has resulted in successful spawning and subsequent wild settlement beyond cultivated areas (Reise 1998; Dutertre et al. 2010;

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

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

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

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

Materials and Methods

Study area and sampling sites

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

Field sampling

Samples of Pacific oysters, mussels and sediments were collected in April 2012. Thirty adult *C.gigas* and *M.edulis* were collected at Mean l~~L~~ow w~~W~~ater from each shore, with fifteen individuals taken in two patches at least 100 m apart. Mussel length was between 25–35 mm, which from population size–frequency histograms would indicate they were at least one year of age (Wright & Bailey, 2009). Oysters sampled were from the most frequent size classes (50–70 mm) which from population size–frequency histograms (McKnight & Herbert, unpublished data) are also estimated to have recruited at least one to two years previously. Samples were frozen at -20 °C in labelled, sealed plastic bags. Data on the size, density and biomass of two intertidal mussel beds sampled in 2008 at Shell Ness and Pegwell (Fig 1) were obtained from Wright & Bailey (2009). These estimates of mean biomass and density were based on monthly grab samples (n =5) of 0.1 m^{-2}. For metal analysis, three surface sediment samples (upper 5 cm) of ~250 g were taken at each site within 1 m of the shellfish samples, placed in a labelled bag and frozen. These samples together with a field assessment of the proportion of gravel, sand, silt and clay at each sampling site were used to classify beach sediments according to Folk (1954).

Laboratory analysis

Defrosted shellfish were opened with a knife to remove the flesh. Oyster and mussel tissues were removed from shells and dried at 60–80°C until constant and mean dry weight was calculated. The dried shellfish were weighed and homogenised separately using a mortar and pestle. Sub-samples of invertebrate material (0.200 g +/- 0.001) were weighed into digestion vessels and 1 ml 37% HCl and 4 ml 70% HNO_3 were added. After microwave digestion (Anton Paar Multiwave 3000; 5 min at 750 W, then 25 min at 100 W; Maichin et al. 2000) samples were filtered through Whatman No. 42 filter papers before being made up to a final volume of 50 ml using deionised distilled water.

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

After microwave digestion (20 minutes at 200 °C) samples were again filtered through Whatman No. 42 filter papers before being made up to a final volume of 50 ml with deionised distilled water. All equipment for digestion and filtering was acid conditioned in 10 % HNO_3. Fisher Primar Plus trace metal grade reagents were used throughout. Metal concentrations in digested samples were determined by inductively coupled plasma–optical emission spectrometry (ICP-OES) for metal analysis (Varian Vista-Pro in axial configuration). Analytical validity was ensured by the digestion and analysis of certified reference materials (NWRI TH-2 harbour sediment) and process blanks. Recoveries were between 70–80% and precision (%RSD) was between 2.6–3.8 (Table S1).

Statistical analysis

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

Results

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

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

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

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

Cadmium

Levels of cadmium did not significantly differ in sediments at the four sites (Table S1). Yet levels were significantly greater in shellfish, except for at Longrock where the difference in levels between sediments and mussels was not statistically significant. There was no significant difference in the levels in Pacific oysters between sampling sites however concentrations were significantly higher than both sediments and mussels at each site. Mean levels in Pacific oysters (2.2 µg g^{-1} dry weight), were over three times that in mussels (0.7 µg g^{-1} dry weight) and at each site were always significantly higher than mussels. Levels in Longrock mussels were significantly less than at other sampling sites.

Lead

Levels of lead in sediments at the two western sites were almost double of those measured at the two eastern sites, with mean levels at Epple Bay the highest (10.9 µg g^{-1} dry weight). In shellfish, mean levels at each site were always less than in the sediments, although at Longrock and Nayland~~s~~ Rock the difference between mussels and sediments was not significant. In contrast to the other metals examined, levels in oysters were always significantly less than in mussels.

Copper

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

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

Zinc

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

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

Discussion

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

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

As the purpose of the investigation was to determine the magnitude of potential metal transfer should the bivalves be consumed by predators within the ecosystem, shellfish were not purged of faeces prior to analysis. Values obtained in this study may therefore be slightly higher than other analyses where bivalves have been purged or undergone depuration. However from estimates of annual biodeposition rates in *C.gigas* (Nishjkawa-Kjnomura, 1978) quantities of Cu and Zn present in the gut is likely to be less than 1% of flesh estimates. Standardisation of metal concentrations to allow for variation in reproductive condition between the two species was not carried out. For mussels, spawning is known to occur during April–May in this region (Wright & Bailey, 2009) and Pacific oysters in summer, assuming sustained temperatures for gametogenesis (Miossec et al. 2009). Bryan et al. (1985) reviewed the influence of sampling period for both species and recommended metal~~s~~ analysis of mussels in late winter to avoid variations in concentration due to food supply and gonad development. In *C.gigas*, lower metal concentrations in winter and summer are attributable to reduced dry mass and dilution due to gonad development, respectively. In field populations of *C.gigas* in the Bay of Bourgneuf (France) concentrations of Cd, Cu Zn, Cd and Pb decreased in the late spring and early summer concomitantly with the increase of soft tissue weight and sexual maturity. Inter-annual variations in concentrations of the metals were also associated with weight changes (Amiard & Berthet, 1996). Therefore, taking account of possible variation in the two species in respect of differing reproductive periods our estimates of metal concentrations are likely to be below annual maximum levels for mussels, whereas estimates in oysters are probably closer to the annual maximum. In the Bay of Bourgneuf, Pb and Cd concentrations in oysters were not correlated with the age or weight; however, Cu and Zn concentrations generally did increase with age (Amiard & Berthet, 1996). Concentrations of Cd and Zn measured in sediments and in *M.edulis* (four size classes, 35 to >50 mm) from two estuaries in s~~S~~outh-east England decreased between December and August in both the years studied (Wright & Mason, 1999). However, overall differences in mean concentrations of Cd and Zn were much greater than variation attributed to shellfish age and sampling season.

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

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

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

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

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

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

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

Conclusion

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

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

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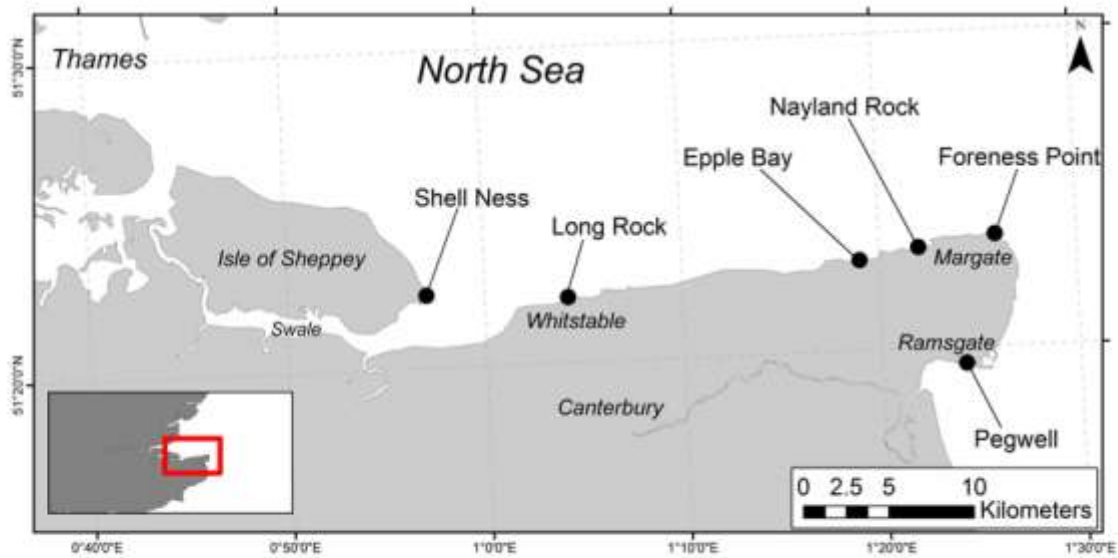

Fig 1. The Thanet coast located in Kent (south-east England), showing mussel and oyster sampling sites at Long Rock, Epple Bay, Nayland Rock and Foreness Point. Estimates of mussel density and biomass were obtained from Shell Ness and Pegwell (Wright & Bailey, 2009). The main port is at Ramsgate.

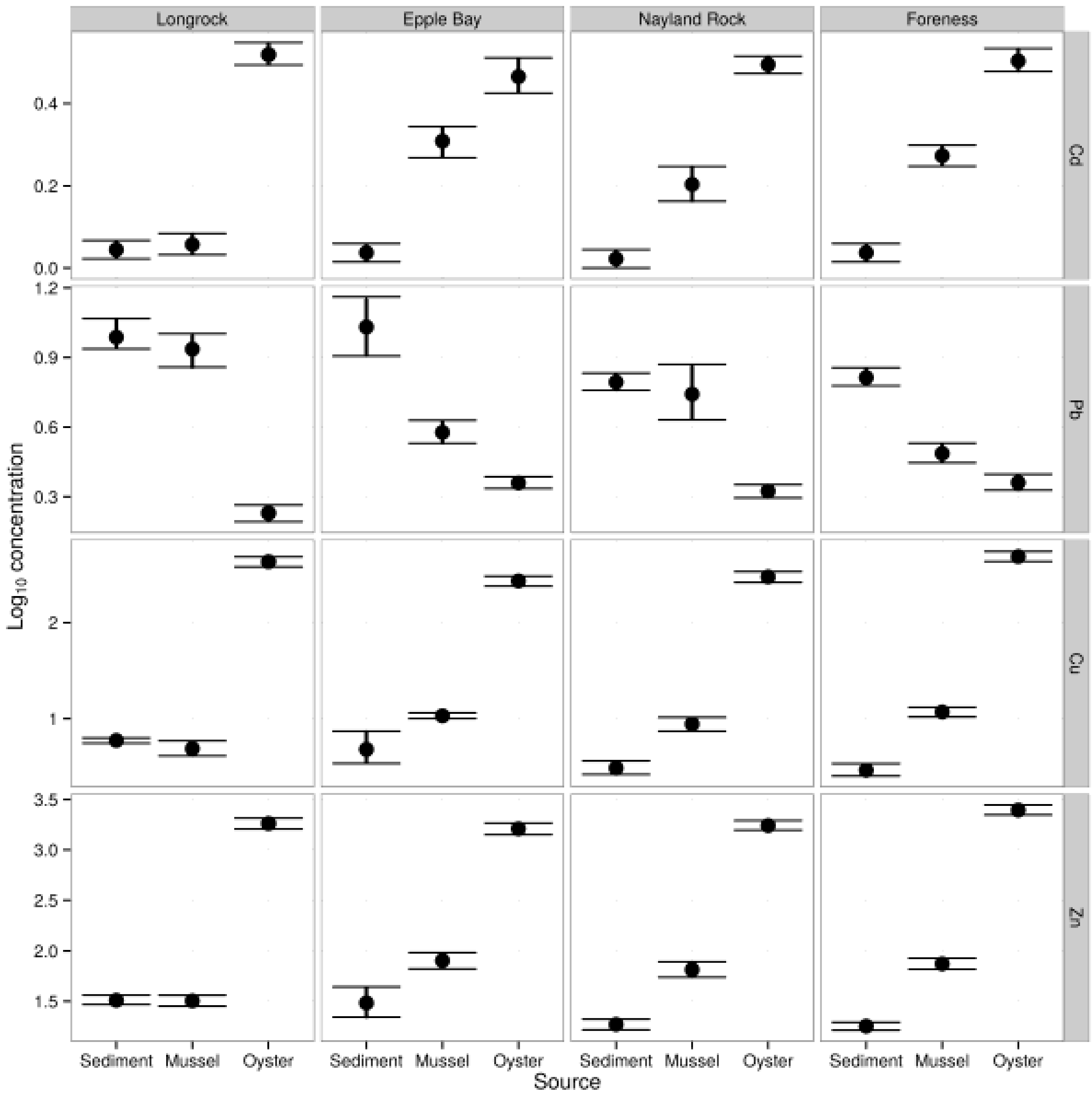

Fig 2. Mean ± 95% Confidence Intervals of metal concentrations (μg g^{-1} dry weight) within sediment and shellfish at sampling sites along the north coast of Kent, UK, in April 2012. Cadmium (Cd); Copper (Cu); Lead (Pb) and Zinc (Zn). Significant differences are apparent for most comparisons between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

Table 1: Mean metal concentrations (µg g^{-1} dry weight) and ±SD (italics) in each source from each shore

	Sediments					***M.edulis***					***C.gigas***				
	Longrock	Epple Bay	Nayland~~s~~ Rock	Foreness	**Mean *(SD)***	Longrock	Epple Bay	Nayland~~s~~ Rock	Foreness	**Mean *(SD)***	Longrock	Epple Bay	Nayland~~s~~ 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)***

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

	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 (*M.edulis*)	0.3	2.3	3.8	30.8
W~~w~~ild Pacific oysters (*C.gigas*)	0.3	0.1	48.5	244.5

Table 3. Mean metal concentration (μg 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. Contaminated samples from Ronce les Bains and the Gironde Estuary were obtained in 1990s (Bragigand et al. 2004, Table 1).

	Mussels (*Mytilus edulis*)				**Pacific oysters (*Crassostrea gigas*)**				
Location	Cadmium μg g^{-1}	Lead μg g^{-1}	Copper μg g^{-1}	Zinc μg g^{-1}	Cadmium μg g^{-1}	Lead μg g^{-1}	Copper μg g^{-1}	Zinc μg g^{-1}	**References**
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 1of 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.

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