Vol. 326: 195–205, 2006 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published November 17

Impact by association: direct and indirect effects of copper exposure on mobile invertebrate fauna

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ABSTRACT: Single-species toxicity studies have been criticised for their inability to test for the effects of pollution on interacting species in field conditions. Pollution may have indirect effects on organisms due to changes in the abundance of competitors, predators or species that act as habitat. In marine hard substrate assemblages, pollution from the heavy metal copper causes predictable changes in the composition of sessile organisms in field conditions. The effects on mobile invertebrates associated with these habitats, however, are unknown. We tested the effects of copper on the entire assemblage of mobile and sessile invertebrates that colonise fouling plates, and demonstrated strong changes in the composition of mobile taxa due to the presence of copper. Manipulative field experiments partitioned the direct effects of water-borne copper from possible indirect effects mediated through changes to their habitat. Sessile assemblages were selectively gardened in the absence of copper to create habitats typical of polluted and unpolluted habitats, and then exposed to copper. The assemblages of mobile invertebrates varied between manipulated habitats, indicating that copper can indirectly affect mobile fauna via habitat change. The mechanisms of these effects were then examined with artificial habitats that mimicked the physical structure of polluted and unpolluted habitats. The composition of the mobile fauna was again dependent on habitat. In both experiments, there were interactions between the effect of habitat and the presence of copper, demonstrating the need for multi-species, field experiments to fully identify the effects of pollutants in natural conditions.

KEY WORDS: Ecotoxicology · Copper pollution · Mobile epifauna · Hard-substrate assemblages · Disturbance · Indirect effects

INTRODUCTION

Understanding the effects of pollution in marine systems is integral to the ongoing management and conservation of marine waterways. The impacts of pollution on marine assemblages have traditionally been assessed and predicted from studies with single species under laboratory conditions (ANZECC & ARMCANZ 2000). This approach has been criticised for its inability to predict the effects of toxicants on entire communities in field conditions (Kimball & Levin 1985, Luoma 1996). The laboratory environment fails to simulate the chemical and biological complexities of toxicants and their interaction with the organisms in the receiving environment (Lewis & Cave 1982, Kimball & Levin 1985, Johnston & Keough 2002, Schiff et al. 2003). These shortcomings have highlighted the need for manipulative field experiments to explore the potential of toxicants to affect entire communities.

Limited field studies have identified both positive and negative effects of toxicants on particular species (e.g. Rygg 1985, Johnston & Keough 2003). Negative effects of pollutants can be explained by direct toxicity; however, positive responses are more difficult to comprehend. While a positive response may indicate that a species has directly benefited from pollution (e.g. if a pollutant, such as sewage effluent, is also a source of food), this is considered unlikely for highly toxic substances. It is more probable that positive responses to pollutants arise from complex indirect effects mediated through associated species (Rygg 1985). Changes in the abundance of a focal species may result from changes in the abundance of that species' predators,

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competitors or those species used as habitat. Such indirect effects are well documented in the marine environment and are acknowledged as an important influence on marine community structure (Menge 1995). Despite their likely importance, there are few studies that have explicitly tested for possible indirect effects of toxicants in any natural ecosystem (see Johnston & Keough 2003 for an exception).

Most sessile marine organisms provide food and/or habitat for a diverse and abundant assemblage of small mobile invertebrates (e.g. seagrass, Jernakoff et al. 1996; macroalgae, Poore et al. 2000; sessile invertebrates, Bradshaw et al. 2003). With changes to the composition and physical structure of such biogenic habitats in the presence of pollution, it is highly likely that pollution can influence mobile invertebrates both directly (via water-borne contaminants) and indirectly through changes to their habitat. Indirect effects are likely as both biological (e.g. species identity, Poore et al. 2000; provision of food, Holloway & Keough 2002) and physical (e.g. habitat complexity, Almany 2004) characteristics of sessile assemblages can strongly affect the abundance and composition of associated mobile invertebrates.

In hard substrate assemblages, manipulative field experiments have found that pollution by the heavy metal copper has both direct and indirect effects on sessile marine invertebrates (Johnston & Keough 2002, 2003). In the presence of copper, the density of solitary ascidians dramatically decreases, while the abundance of serpulid polychaetes greatly increases. The increase in serpulids in the presence of copper was an indirect effect of the pollutant, mediated through competition for space (Johnston & Keough 2002, 2003). Given that these sessile invertebrates are also habitat for a diverse array of small, mobile invertebrates, the demonstrated effects of copper on sessile assemblages may have further consequences for the ecology of the entire assemblage.

Despite the likelihood of pollution affecting mobile invertebrates indirectly (via changes to the habitat), no field experiments have examined both direct and indirect effects of a toxicant on an entire assemblage of mobile invertebrates. To address this shortcoming, we aim to test the relative importance of direct and indirect effects of copper pollution on the mobile invertebrates associated with hard-substrate sessile assemblages. We asked 3 specific questions: (1) Are natural assemblages of mobile invertebrates affected by copper pollution? (2) Are any changes in the assemblage of mobile invertebrates in the presence of copper caused by the toxicant directly, or indirectly by changes in the associated sessile fauna? (3) Are any indirect effects on the mobile fauna due to changes in the physical structure of the habitat alone?

MATERIALS AND METHODS

Study site and organisms. The 2 study sites used for these experiments were Kurnell Pier in Botany Bay (34° 00′ 15″ S, 151° 12′ 42″ E) and Chowder Bay in Port Jackson (33° 50′ 23″ S, 151° 15′ 10″ E), both in the Sydney region, Australia. Heavy metal concentrations in the water and sediments are negligible at both sites (Birch 1997, Piola & Johnston 2006). In temperate regions on the east coast of Australia, hard-substrate fouling organisms typically consist of species of encrusting and arborescent bryozoans, sponges, colonial and solitary ascidians, filamentous and foliose algae, barnacles and tube-dwelling polychaetes (Glasby 1997). These organisms readily settle on artificial substrates (e.g. perspex plates). The mobile invertebrates associated with these sessile organisms include gammarid and caprellid amphipods, isopods, mobile polychaetes, gastropods and harpacticoid copepods (see ‘Results’).

Effects of copper on entire hard substrate assemblages. A contrast of the assemblages that formed in the presence of copper-based antifouling paint with those in the absence of copper was used to test the effects of copper on the abundance and composition of both sessile and mobile fauna simultaneously. International Micron Extra® copper-based antifouling paint was used as the reference toxicant in this and subsequent experiments, and is recognised as a relevant and effective field-based dosing system (Webb & Keough 2002, Piola & Johnston 2006).

Sixteen black perspex settlement plates (11 × 11 cm) were used as the replicate habitat units on which organisms were allowed to settle. Prior to use, all settlement plates were acid washed in 5% nitric acid for a minimum time of 48 h and then rinsed with freshwater (APHA 1995). A 2.5 cm wide border of International Interprotect® epoxy primer was painted around the outside of all plates, leaving 6 × 6 cm of unpainted substrate in the centre of the plate for settlement. Eight plates were dosed with copper by painting 2 coats of copper antifouling paint over the primer, while 8 control plates received no antifouling paint. To prevent cross-contamination between the copper and control treatments, plates were individually bolted inside 2 l plastic containers. Each replicate was then bolted onto the underside of two 60 × 60 cm PVC backing plates. The copper treatments and controls were randomly dispersed among and within the 2 backing plates. All fasteners were made of marine-grade stainless steel to minimize the potential effects of metal pollution from corrosion.

The plates were suspended horizontally at a depth of approximately 3 m below the low water mark at Kurnell Pier, and remained submerged for 4 mo.

The painted borders of copper paint and control primer were wiped with scourers every few weeks to remove developing biofilms and to ensure the continued release of copper ions. After 4 mo, the plates were collected using SCUBA. To minimise disturbance to the mobile fauna, one diver held the backing panel steady while a second diver carefully sealed a lid onto each 2 l container. The plates were therefore completely enclosed before being carefully extracted from the water and brought back to the lab. All organisms associated with the plates were preserved with 5% formaldehyde.

Samples were rinsed and passed through a 300 µm sieve to retain mobile invertebrates. Using a dissecting light microscope, all individuals were identified and counted within the following taxonomic groups: gammarid amphipods, caprellid amphipods, isopods, polychaetes, copepods, gastropods and ostracods. Once counted, samples were transferred to 70% ethanol for long-term storage. The abundance of sessile organisms on the plates was recorded as percentage cover for colonial ascidians and bryozoans, and as counts per plate for barnacles, serpulid worms and solitary ascidians. Barnacles were counted in 2 size classes; small (<4 mm) and large (>4 mm) due to very large number of recently recruited barnacles that did not take up a substantial amount of settlement space.

The difference in composition of mobile taxa between the copper and control treatments was visualised using an MDS ordination, and analysed using permutational multivariate analysis of variance (PERMANOVA; Anderson 2001, available at www.stat.auckland.ac.nz/~mja). Separate analyses were conducted with the sessile organisms to confirm predicted changes in habitat. The abundances of the most commonly collected taxa were contrasted between copper and control treatments with *t*-tests.

Experimental manipulation of sessile assemblages. The effects of copper on mobile invertebrates (see 'Results') could arise from direct effects of the water-borne copper or indirect effects mediated through changes to the sessile assemblages. To separate these direct and indirect effects of copper, we contrasted the effect of copper on mobile invertebrates that colonised the following 3 habitat treatments: (1) assemblages manipulated to resemble those found at polluted sites (2) assemblages manipulated to resemble those found at unpolluted sites, and (3) unmanipulated assemblages.

Thirty-six settlement plates (6 × 6 cm) were suspended horizontally under Kurnell Pier for 4 mo. During this time, habitat manipulations were made by selectively gardening organisms approximately every 2 wk to achieve a serpulid-dominated habitat (to simulate a polluted habitat), or an ascidian-dominated habitat (to simulate an unpolluted environment). There were 12 plates in each manipulated treatment, and 12 as an unmanipulated control. All habitat manipulations were done in the absence of copper. After 4 mo, when the sessile assemblages had established and there were clear differences between the habitat treatments, copper antifouling paint was added to half the plates within each habitat treatment. Individual plates were bolted onto a larger (11 × 11 cm) plate, which was prepared as either a copper treatment using anti-fouling paint or as a control using epoxy primer (as described above). To prevent cross-contamination, each replicate was placed inside a 2 l plastic container and bolted onto one of 3 backing panels. Plates were maintained in a pool of local seawater to prevent desiccation during this procedure prior to their resubmergence. There were 6 replicate plates in each combination of the copper and habitat treatments. Treatments were randomly interspersed on backing plates.

After 5 d, the plates were collected and the mobile and sessile invertebrates counted using the procedures as described above; 5 d was found to be sufficient time for the colonisation of mobile invertebrates without changes in the cover of sessile organisms due to the presence of copper (see 'Results: Experimental manipulation of sessile assemblages'). The differences in the composition of mobile and sessile invertebrate taxa between the treatments were visualized by MDS ordination plots and analysed by a 2-factor PERMANOVA with copper and gardening treatments as fixed factors. The abundance of the most commonly collected mobile and sessile taxa were contrasted among treatments using 2-factor ANOVA.

Effects of copper in artificial habitats. Indirect effects of copper on the mobile fauna, mediated through changes to the sessile assemblages, could arise due to changes in the species composition of the sessile organisms, or simply changes to the physical structure of the habitat. To isolate whether the physical structure of habitat alone affects mobile fauna, we used artificial assemblages to mimic the physical structure of those assemblages found in polluted and unpolluted sites. The copper treatment was applied to each type of artificial habitat in a factorial design.

Artificial habitats that mimicked the serpulid-dominated habitats occurring at a polluted site were created by cutting up plastic into serpulid-sized fragments approximately (0.3 × 2 × 0.1 cm), while ascidian-dominated habitats mimicking unpolluted sites were created with life-size plastic grapes and a few serpulid fragments. The habitat structure was consistent among replicates within each mimic treatment. Sixteen mimic habitats for each treatment were constructed on 6 × 6 cm perspex plates. The plates were each bolted onto 11 × 11 cm plates, half treated with copper paint and half with epoxy primer as a control. There were 8 replicates for each combination of the habitat and copper treatments. Each plate was then placed inside 2 l containers and deployed as described above. The panels were submerged in approximately 3 m of water from the shark net at Chowder Bay. This site enabled plates to be suspended vertically against a substrate containing a well-established assemblage of mobile and sessile invertebrates. Such placement is thought to mediate the rapid recruitment of mobile invertebrates (Glasby & Connell 2001). Pilot studies had shown relatively few mobile invertebrates colonising the artificial habitats at Kurnell Pier over short time scales.

After 2 wk, the plates were collected and the mobile invertebrates were counted using the procedures as described above. The duration of the experiment was longer than the 5 d exposure above as pilot studies had shown that a 5 d period was insufficient to attract large numbers of invertebrates to the artificial habitats. The differences in the composition of mobile taxa between the treatments were visualized by MDS ordination plots and analysed by a 2-factor PERMANOVA with copper and habitat as fixed factors. The abundances of the most commonly collected mobile taxa were contrasted among treatments using 2-factor ANOVA with copper and habitat as fixed factors.

Statistical analyses. Multivariate analyses were performed using PERMANOVA (Anderson 2001). The Bray-Curtis dissimilarity measure was used as the distance metric with 9999 permutations for the probability tests. Data for sessile organisms were standardised to equal abundance per sample as some variables were counts and others percentage cover. MDS ordination plots were created using Bray-Curtis similarities and the software Primer (Clarke & Warwick 1994). Univariate *t*-tests and ANOVA were performed using SYSTAT 10 (SPSS, 2000). Data were assessed for normality and homogeneity of variance using frequency histograms of residuals, and plots of residuals versus means, respectively. When required, arcsine (square-root), log, or square-root transformations were applied.

RESULTS

Effects of copper on entire hard substrate assemblages

Contamination of hard substrate assemblages by copper caused the predicted changes in the composition of sessile (habitat forming) organisms (Fig. 1a, PERMANOVA, $F = 5.25$, df = 1,14, $p < 0.001$). Copper increased the number of serpulids ($t = 2.34$, df = 14, $p = 0.035$) and small barnacles ($t = 2.69$, df = 14, $p = 0.018$), and reduced the cover of bryozoans ($t = 1.19$, df = 14, $p = 0.005$, arcsine transformed data) and the number of large barnacles ($t = 4.14$, df = 14, $p = 0.001$). On the copper-treated plates, the counts of solitary ascidians and cover of colonial ascidians decreased in comparison to the control plates but these differences were not significant ($t < 1.18$, df = 14, $p > 0.1$). Overall recruitment of sessile invertebrates was reduced in the presence of copper, with a greater area of the settlement plates bare (40% vs. 3% in controls, $t = 4.18$, df = 14, $p < 0.001$).

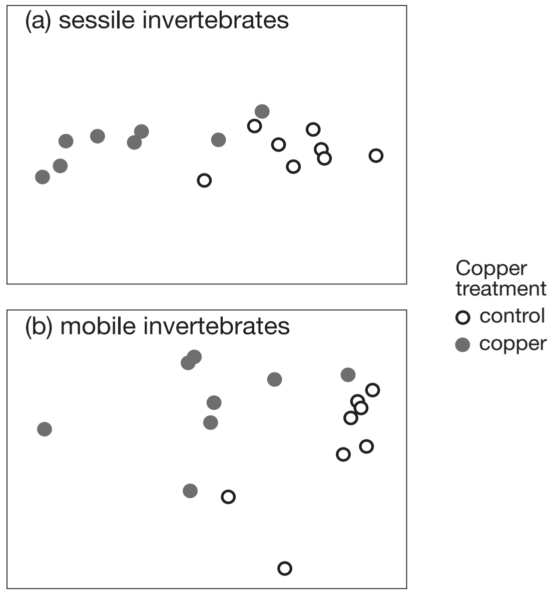

Fig. 1. MDS ordination contrasting invertebrate assemblages in the presence and absence of copper. Ordination of (a) sessile fauna based on counts per plate or percentage cover of all major taxonomic groups recorded, with data standardised to equal abundance per plate (stress = 0.03); ordination of (b) mobile fauna based on counts per plate of major taxonomic groups (stress = 0.05). Both conducted with the Bray-Curtis similarity index

The composition of the mobile invertebrate assemblage associated with the sessile invertebrates varied between the copper and control treatments (Fig. 1b, PERMANOVA, $F = 10.15$, df = 1,14, $p < 0.001$). Gammarid amphipods were the most abundant mobile invertebrates, with almost half as many on the copper plates compared to the control plates (Fig. 2a, $t = 3.70$, df = 14, $p = 0.002$). The abundance of mobile polychaete worms was also greatly reduced by the presence of copper (Fig. 2b, $t = 3.41$, df = 14, $p = 0.004$). In contrast, caprellid amphipods were more abundant on the copper treated plates than on the control plates (Fig. 2c, $t = 3.12$, df = 14, $p = 0.008$). There were no treatment effects on the abundance of copepods and gastropods (Fig. 2d,e, $t < 1.55$, df = 14, $p > 0.15$).

Experimental manipulation of sessile assemblages

The selective removal of sessile organisms successfully created 2 distinct habitats in addition to the un-

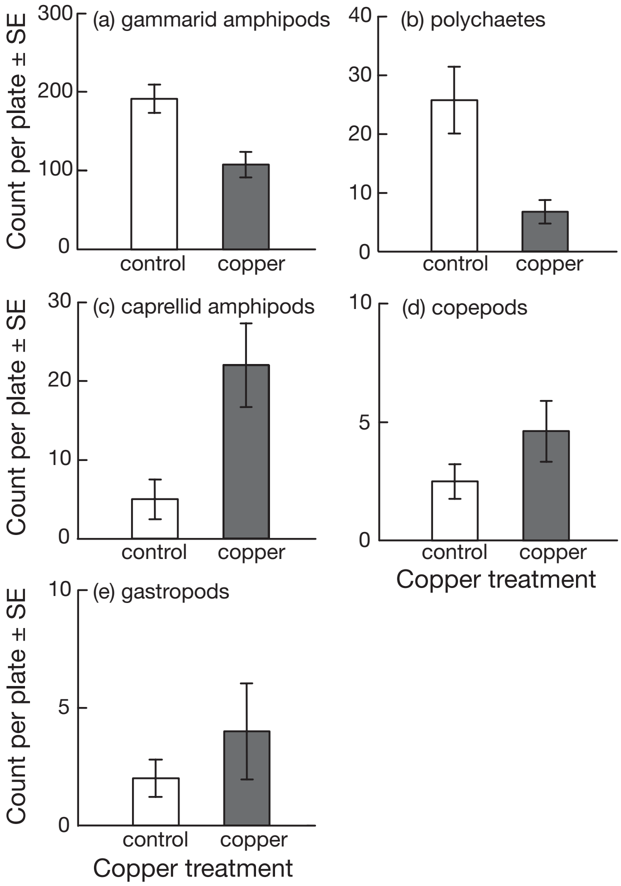

Fig. 2. Effect of copper on abundance of mobile invertebrates inhabiting sessile assemblages. Data are mean counts per plate (±SE) of: (a) gammarid amphipods, (b) polychaetes, (c) caprellid amphipods, (d) copepods and (e) gastropods (n = 8 per treatment)

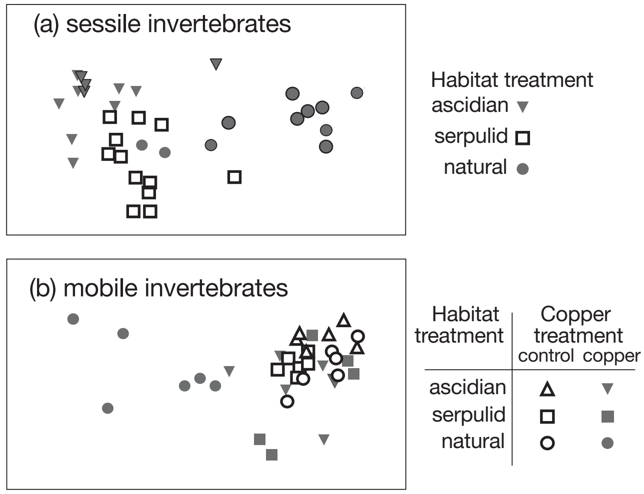

Fig. 3. MDS ordination contrasting invertebrate assemblages in the presence and absence of copper across 3 habitat treatments; (1) ascidian dominated, (2) serpulid dominated, and (3) unmanipulated. Ordination of (a) sessile fauna based on counts per plate or percentage cover of all sessile major taxonomic groups recorded with data standardized to equal abundances per plate (stress = 0.07); ordination of (b) mobile fauna based on counts per plate of major taxonomic groups (stress = 0.08). Both ordinations conducted with Bray-Curtis similarity index

manipulated control (Fig. 3a, Table 1, all levels of the habitat treatment differed in pairwise tests following PERMANOVA). The manipulations resulted in an abundance of serpulids in the serpulid-dominated habitat approximately 5 times that of the controls (F = 28.56, df = 1,30, p < 0.001). The habitat manipulation did not successfully increase the abundance of colonial ascidians in the ascidian-dominated habitat (F = 2.86, df = 1,30, p = 0.91). Solitary ascidians recruited in very low numbers and were not analysed. The 2 manipulated habitats had lower abundance of large barnacles (habitat treatment, F = 45.25, df = 1,30, p < 0.001) and a higher cover of bare space (F = 12.06, df = 1,30, p < 0.001) relative to the unmanipulated controls. The abundance of small barnacles and the percentage cover of bryozoans did not differ among the habitat treatments (F < 3.63, df = 1,30, p > 0.06). The 5 d exposure to copper at the end of this experiment caused no changes to the abundance of any sessile taxa (copper treatment, F < 0.32, df = 1,30, p > 0.58 for all tests). There were no significant interactions between the habitat and copper treatments with the exception of serpulids (F = 14.52, df = 1,28, p < 0.001). Tukey's post-hoc tests on the abundance of serpulids confirmed that short-term exposure to copper did not affect their abundance in any of the 3 habitats, and that they were significantly more abundant in the serpulid-dominated than the ascidian-dominated habitat.

Table 1. Two-factor PERMANOVA contrasting the composition of (a) sessile invertebrates and (b) mobile invertebrates among habitat treatments in the presence or absence of copper (n = 6 per combination of treatments). Analysis of sessile fauna based on counts per plate or percentage cover of all major taxonomic groups recorded, with data standardised to equal abundance per plate. Analysis of mobile fauna based on counts per plate of major taxonomic groups with data square-root transformed and using the Bray-Curtis dissimilarity index. *Significant result (p < 0.05)

Source	df	(a) Sessile invertebrates			(b) Mobile invertebrates		
		MS	F	p	MS	F	p
Copper	1	1009.53	0.77	0.61	1742.74	5.97	<0.001*
Habitat	2	8622.42	6.55	<0.001*	1120.43	3.84	<0.001*
Copper × Habitat	2	885.47	0.67	0.79	1473.73	5.05	<0.001*
Error	30	1316.30			291.89		

The composition of the mobile invertebrate assemblage was strongly dependent on both habitat and the presence of copper, with the 2 factors interacting to determine assemblage structure (Fig. 3b, Table 1, all levels of the habitat treatment differed in pairwise tests following PERMANOVA) and the abundance of individual taxa (gammarid and caprellid amphipods, copepods, Fig. 4). Gammarid amphipods were the most abundant mobile invertebrates, with abundances reduced in the presence of copper in the natural and ascidian-dominated habitats, but not the serpulid-dominated habitat (Fig. 4a, Table 2a). In contrast, the abundance of caprellid amphipods was significantly higher in the presence of copper in the natural assemblage, and unaffected by copper in the 2 gardened assemblages (Fig. 4b, Table 2b). Copper reduced the abundances of copepods in the natural assemblage and had no effect in the 2 gardened assemblages (Fig. 4c, Table 2c). Neither habitat nor copper affected the abundance of mobile polychaete worms (Fig. 4d, Table 2d).

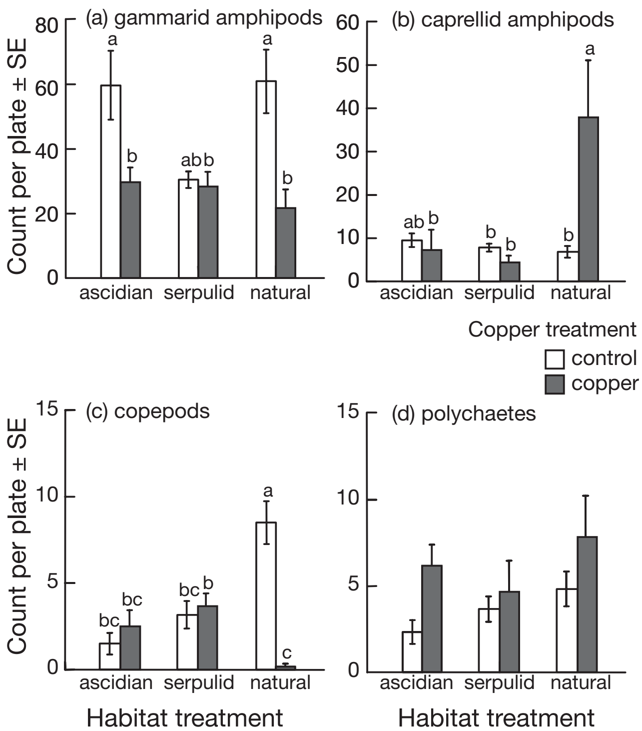

Fig. 4. Effect of copper on the abundance of mobile invertebrates across 3 habitat treatments; (1) ascidian dominated, (2) serpulid dominated, and (3) unmanipulated. Data are mean counts per plate (±SE) of: (a) gammarid amphipods, (b) caprellid amphipods, (c) copepods and (d) polychaetes (n = 6 per treatment). For taxa displaying significant interaction between habitat and copper treatment, bars sharing the same letter do not differ significantly in Tukey's post-hoc tests

Effects of copper in artificial habitats

The artificial habitats were colonized by an abundant assemblage of mobile invertebrates. The composition of this assemblage was again strongly dependent on both habitat and the presence of copper. These factors, however, did not interact to determine assemblage structure (Fig. 5, Table 3). Gammarid amphipods were again the most abundant taxon, with lower abundance in the presence of copper (reduced by approximately 60 %) and on the artificial serpulid habitat (Fig. 6a, Table 4a). The abundance of isopods was similarly affected by copper,

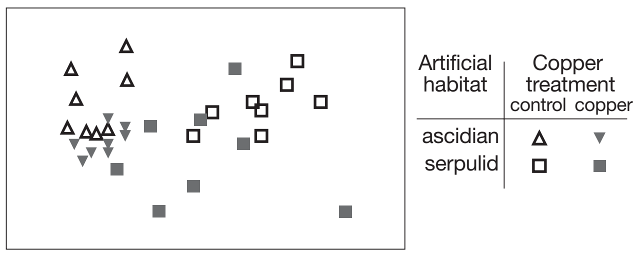

Fig. 5. MDS ordination contrasting mobile invertebrate assemblages in the presence and absence of copper in 2 artificial habitats. Habitats mimic either an ascidian-dominated habitat or a serpulid-dominated habitat. Ordination based on counts per plate of major taxonomic groups and conducted with Bray-Curtis similarity index (stress = 0.09) (n = 8 per treatment)

Table 2. Two-factor ANOVA contrasting abundance of (a) gammarid amphipods, (b) caprellid amphipods, (c) copepods, and (d) polychaetes among habitat treatments in the presence and absence of copper (n = 6 per combination of treatments). Data for (a), (b) and (d) were log transformed *Significant result ($p < 0.05$)

Source	df	(a) Gammarid amphipods			(b) Caprellid amphipods			(c) Copepods			(d) Polychaetes		
		MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p
Copper	1	3.73	24.44	<0.001*	0.01	0.02	0.89	46.69	14.37	<0.001*	0.60	1.88	0.17
Habitat	2	0.34	2.21	0.13	3.03	5.90	0.01*	16.58	5.10	0.01*	1.28	4.05	0.05
Copper × Habitat	2	0.77	5.01	0.01*	4.79	9.32	<0.001*	82.69	25.44	<0.001*	0.53	1.67	0.21
Error	30	0.15			0.51			3.25			0.32		

but there were no differences between the 2 habitats (Fig. 6b, Table 4b). The copper and habitat treatments interacted to determine the abundance of copepods, with the 2 habitats only differing in the absence of copper (Fig. 6c, Table 4c). Gastropods and polychaetes were unaffected by copper, and both were more abundant on the mimic ascidian habitat than on the mimic serpulid habitat (Fig.6d,e, Table 4d,e).

Table 3. Two-factor PERMANOVA contrasting composition of mobile invertebrates between artificial habitat treatments in the presence and absence of copper (n = 8 per combination of treatments). Analysis based on counts per plate of major taxonomic groups with data square-root transformed and using the Bray-Curtis dissimilarity index. *Significant result (p < 0.05)

Source	df	MS	*F*	p
Copper	1	2771.02	17.83	<0.001*
Habitat	1	1135.89	7.31	<0.001*
Copper × Habitat	1	265.57	1.709	0.15
Error	8	155.37		

DISCUSSION

Effects of pollutants on mobile invertebrates

This study is the first to reveal that pollution from water-borne copper alters the mobile invertebrate assemblages associated with hard substrates. Although many ecotoxicological studies have demonstrated that pollutants can affect mobile invertebrates, these studies have generally been confined to soft sediments (e.g. Stark 1998, Trannum et al. 2004) or single-species laboratory studies (e.g. Schratzberger et al. 2002). The

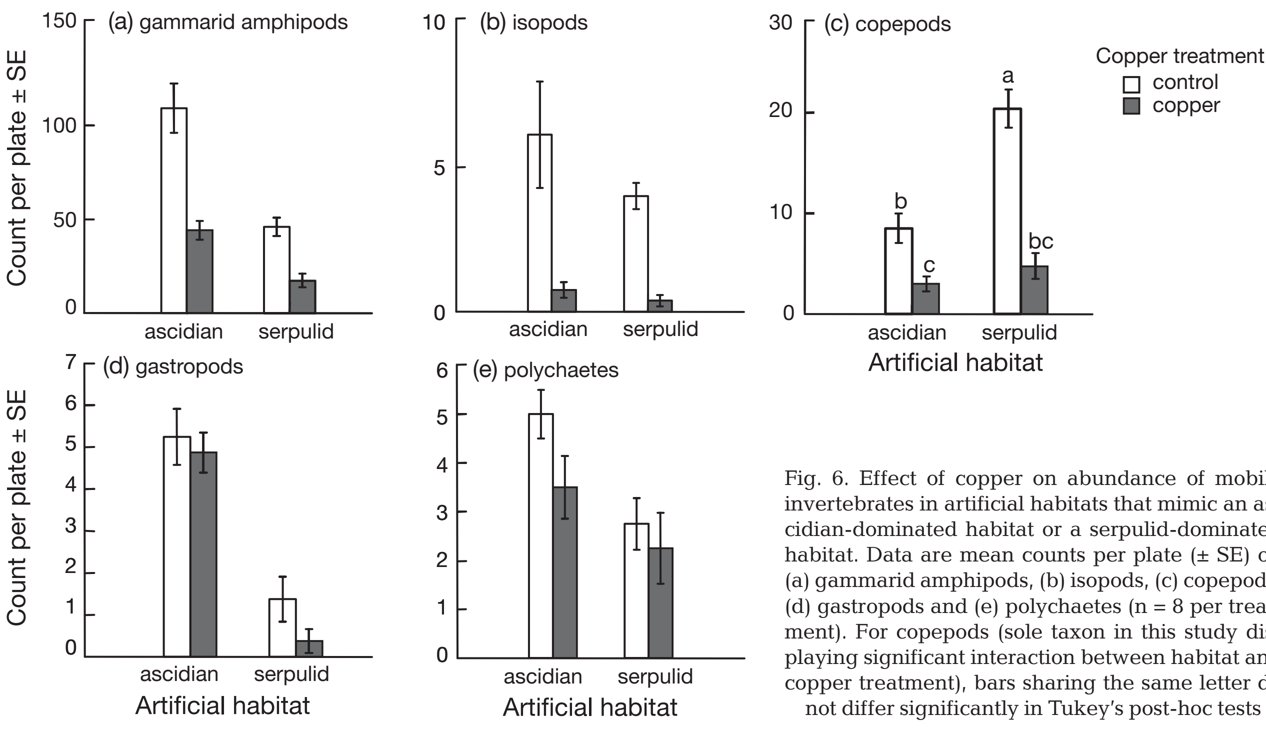

Fig. 6. Effect of copper on abundance of mobile invertebrates in artificial habitats that mimic an ascidian-dominated habitat or a serpulid-dominated habitat. Data are mean counts per plate (± SE) of: (a) gammarid amphipods, (b) isopods, (c) copepods, (d) gastropods and (e) polychaetes (n = 8 per treatment). For copepods (sole taxon in this study displaying significant interaction between habitat and copper treatment), bars sharing the same letter do not differ significantly in Tukey's post-hoc tests

Table 4. Two-factor ANOVA contrasting abundance of (a) gammarid amphipods, (b) copepods, (c) isopods, (d) gastropods and (e) polychaetes between artificial habitat treatments in the presence and absence of copper (n = 8 per combination of treatments). Data for (a) were log transformed. *Significant result (p < 0.05)

Source	df	(a) Gammarid amphipods			(b) Isopods			(c) Copepods			(d) Gastropods			(e) Polychaetes		
		MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p
Copper	1	7.92	53.91	<0.001*	162	25.45	<0.001*	892.53	63.21	<0.001*	3.78	2.11	0.16	8.00	3.16	0.09
Habitat	1	7.24	49.28	<0.001*	12.5	1.96	0.17	371.28	26.29	<0.001*	140.28	78.36	<0.001*	24.50	9.66	0.004*
Copper × Habitat	1	0.06	0.41	0.53	6.13	0.96	0.34	205.03	14.52	<0.001*	0.78	0.44	0.51	2.00	0.79	0.38
Error	28	0.15			6.37			14.12			1.79			2.536		

marine assemblage investigated in this field study is characteristic of temperate, hard-substrate, shaded communities dominated by ascidians, bryozoans, barnacles and serpulids (Glasby & Connell 2001). The associated mobile assemblage was dominated by gammarid amphipods, with harpacticoid copepods, caprellid amphipods, gastropods and mobile polychaetes present in lower abundances. Complex interactions between the sessile and mobile communities greatly influenced the effect of pollution on the entire assemblage.

Ecotoxicological field studies on marine sessile communities have found that pollution dramatically alters community composition (e.g. Johnston & Keough 2002, 2003). In our study, the composition of the sessile marine assemblages was similarly affected by water-borne copper. Clean habitats were dominated by colonial ascidians and large barnacles, while polluted habitats were dominated by serpulids and small barnacles. This is remarkably consistent with the effect of copper pulses at a site in Port Philip Bay, Victoria, Australia (Johnston & Keough 2002). The compositional change in sessile fauna alters the structural complexity of the habitat with more uncolonised space present in polluted habitats.

In addition to changing the composition of sessile assemblages, exposure to copper dramatically altered the abundance of associated mobile invertebrates. Some mobile invertebrates were disadvantaged by copper pollution (gammarid amphipods and mobile polychaetes), some were advantaged by copper pollution (caprellid amphipods) while others showed no effect of copper pollution (gastropods). The range of effects of copper on different groups of mobile fauna cannot be explained by a single mechanism. Negative effects may be due to direct toxicity, or behavioural avoidance of contaminated sites (Wiklund et al. 2006). Many studies on single species have demonstrated the toxicity of copper to marine invertebrates, including amphipods (e.g. Reish 1993), copepods (e.g. Hall et al. 1997) and polychaetes (e.g. Rees 1983). While the concentration of copper ions released from the antifouling paint was not measured in this study, past analyses using a similar dosing technique found copper concentrations ranging between 7 and 120 $\mu g\ l^{-1}$ (Webb & Keough 2002). The large range of values was due to variations in site and season, yet even the lowest values are high enough to cause chronic toxicity to a number of taxa, and acute toxicity to some extremely sensitive species (Hall & Anderson 1999). Behavioural avoidance of plates dosed with copper is also likely, with many studies showing that the epifauna associated with sessile marine organisms is highly mobile (e.g. Martin-Smith 1994, Poore 2004), Such mobility will allow animals to depart contaminated sites, or select against such sites when colonising new habitats. Differences among taxa in the direct responses to copper may relate to differential mobility, and/or sensitivity.

While caprellid amphipods are highly mobile (Martin-Smith 1994), and potentially able to avoid contaminated substrates, their abundance increased in the presence of copper. The positive response of caprellid amphipods indicates an indirect effect of copper and at least some tolerance to this metal. Tolerance to copper is achieved through various detoxification and excretory processes and has been observed in many marine invertebrates in the past, including crustaceans (Correia et al. 2002, Marsden & Rainbow 2004). We know of no ecotoxicological studies involving copper and caprellid amphipods. The response of some caprellid species to tributyltin has been studied, with their ability to degrade TBT suggested to be lower than that of gammarid amphipods (Ohji et al. 2002, 2005). Even if the caprellids studied here were tolerant of copper, it is unlikely that such a highly-toxic substance would directly benefit these species (Rygg 1985). Positive effects of toxic pollutants are far more likely to arise due to complex indirect effects.

Copper could indirectly affect mobile invertebrates through changes to the associations among mobile species, and via changes to the habitat. Negative correlations between the abundance of caprellid and gammarid amphipods in both the presence and absence of copper in the natural assemblages suggest interspecific competition between these taxa. Such associations between mobile fauna are poorly understood. While marine epifaunal assemblages can be food-limited in both algal (Edgar & Aoki 1993) and seagrass (Edgar 1990) systems, and thus may compete for food, there is as yet no strong evidence of competition between associated mobile invertebrates (Gunnill 1984, Poore et al. 2000).

Effects of pollution mediated through changes in habitat

Biogenic habitats influence their associated mobile fauna both physically and biologically (Kelahar 2002), so any change in their composition or structure due to pollution is likely to influence the array of associated mobile organisms. The gardening and artificial habitat manipulations partitioned the effects of water-borne copper pollution from the indirect effects of habitat change. The gardening manipulation successfully created 2 distinct habitats. The gardened polluted habitat successfully mimicked copper polluted assemblages as it was dominated by serpulids (Johnston & Keough 2000, 2002). Serpulids are good colonisers but poor competitors (Kay & Keough 1981), so freeing up bare space often initiates a positive response from these organisms. The gardened unpolluted habitat was also dominated by similar species to those that naturally occur on shaded unpolluted hard-substrata (e.g. colonial and solitary ascidians; Johnston & Keough 2000, 2002).

Distinct assemblages of mobile invertebrates between the 2 gardened habitats revealed for the first time that copper pollution indirectly affects mobile fauna, with the indirect effects mediated through changes in habitat composition. Gammarid amphipods responded negatively to copper exposure directly as well as indirectly through pollution-induced habitat change. Such marked negative effects of pollution could have wide implications, as gammarids are highly abundant in most marine habitats in temperate Australia (e.g. algal beds and sponge beds, Poore et al. 2000) and are an important food source for many fish. Caprellid amphipods again displayed a positive response to the toxicant, with no evidence of an indirect effect mediated through habitat. Further work is required to explain the generally positive response of caprellids to this highly toxic substance. Species-level discrimination of such samples would aid in identifying tolerant species and contribute to a further understanding of community level responses to toxicants.

While the gardened habitats were made up of sessile species that are found in polluted and unpolluted sites, the gardening manipulation was unable to separate the physical and biological influence of habitats on their associated mobile invertebrates. The use of artificial habitats allowed a test of the importance of the physical structure of these habitats alone on the associated mobile invertebrates. While artificial habitats are only representative of their natural counterparts, they have been widely used to test hypotheses about the distribution of mobile invertebrates (e.g. Dean 1981, Edgar 1991, Kelahar 2002), due to their ease in providing replicate substrates of known physical structure (Glasby & Connell 2001). This is the first time that artificial habitats have been specifically designed to mimic polluted and unpolluted habitats. This habitat manipulation again demonstrated that the changes to habitat as expected from exposure to copper will strongly affect the associated mobile fauna. In this case, these indirect effects of pollution are clearly mediated through the physical structure of the habitat alone rather than its biological composition. Most taxa (gammarid amphipods, isopods, gastropods and polychaetes) were more abundant on the physical structure associated with unpolluted habitats. These results are consistent with many that demonstrate increased invertebrate abundance in habitats of greater structural complexity (e.g. Connolly 1997, Almany 2004).

In contrast to these taxa, copepods were most abundant in the artificial polluted habitat, but only in the absence of copper. The interactive effects of copper and habitat in determining animal abundance in this and the previous experiment highlight the complex effects of pollution on sessile marine communities. The effects of copper on gammarid and caprellid amphipods in the gardening experiment, and on copepods in both manipulative experiments, were dependent on habitat types. Differences in the responses of taxa across experiments also indicate that toxicant effects are not easily predicted in field conditions.

CONCLUSIONS

Copper-based antifouling paint dramatically altered the abundance and composition of hard-substrate fouling assemblages characteristic of ports and harbours in temperate regions. These assemblages are commonly exposed to antifouling paints through shipping traffic and following the banning of tributyltin, the use of copper-based antifouling paints is set to increase (IMO 2001). The direct and indirect effects of copper demonstrated in this study can potentially be observed in a large proportion of ports and harbours in temperate regions of the world. This has broad implications, as mobile invertebrates are a significant component of local biodiversity and an important source of food for many species of fish.

The prevalence of interactions between a toxicant and its receiving habitat emphasises the complexities of natural systems and highlights the need for manipulative field ecotoxicological studies to clarify the actual effects of pollution on natural marine systems. Without field studies, indirect effects of pollutants can be neither detected nor understood (DeAngelis 1996, Johnston & Keough 2003). The complex effects of copper on hard-substrate assemblages and their associated mobile invertebrates, as detailed in this study, suggest that there will be indirect effects of disturbances such as pollution in other habitats where mobile fauna are associated with biogenic habitats. There is a diverse array of mobile fauna associated with a host of biogenic habitats including macroalgae (e.g. Poore et al. 2000), seagrass beds (e.g. Edgar 1992) and mussel beds (Chapman et al. 2005). The protection of this component of marine biodiversity requires an understanding of both the direct impacts of pollution and the indirect effects resulting from changes to the biogenic habitats on which they depend.

Acknowledgements. We thank B. Cumbo, C. Barclay, K. Monro, N. Hill, R. Piola, D. Roberts, K. Dafforn and K. Stuart for assistance in the field, and K. Monro and I. Suthers for comments that have improved this manuscript. We are grateful to L. Hughes for her assistance in the development of artificial assemblages, and to K. Gotham for providing access to Caltex Pier. This research was supported by Australian Research Council Discovery Projects DP0556372 to A.G.B.P. and DP0452495 to E.L.J.

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Editorial responsibility: Howard Browman (Associate Editor-in-Chief), Storebø, Norway

Submitted: March 23, 2006; Accepted: June 12, 2006
Proofs received from author(s): October 31, 2006