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A widespread contaminant enhances invasion success of a marine invader

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Summary

1. The global transfer of species by human vectors is continuing despite the use of managerial controls such as antifouling biocides and pesticide applications. The process of introduction now exposes species to novel conditions which may select for tolerance to a contaminant. Invader establishment success is influenced by both the supply of invasive propagules and disturbance. Therefore, it is important to understand whether tolerance to an anthropogenic disturbance, such as contamination, can change the parameters of supply in a way that inadvertently augments the invasion process.

2. To test whether the invasion process is influenced by a widespread contaminant, we investigated how recruitment of the invasive hull-fouling bryozoan *Watersipora subtorquat*a is affected by exposure to copper-based antifouling paint. We quantified settlement patterns in control and copper environments and then assessed post-settlement survival and fitness components.

3. Copper significantly increased total recruitment success despite greater post-settlement mortality. Surviving recruits differed morphologically, with shorter ancestrulae and smaller colonies in high copper treatments. These results show a strong positive affiliation between larval *W. subtorquata* and high levels of copper although there are associated fitness costs.

4. *Syntheses and applications*. We found a direct positive effect of contamination on recruitment of a common invasive species. This process is likely to be relevant to other non-indigenous species (NIS) that exhibit a positive affiliation with metal contamination. Copper can potentially enhance success at multiple stages of the invasion process, including facilitating transport and establishment, by increasing the supply and retention of individuals into anthropogenically disturbed environments. Identification of tolerance to contamination as a species trait may also aid in predicting a species invasiveness and spread. Management of metal pollution through remediation and alternative copper-free antifouling techniques would help prevent the spread and establishment of many marine NIS.

Key-words: Bryozoans, contamination, copper, metamorphosis, non-indigenous species, propagule pressure, recruitment, trade-off

Introduction

Anthropogenic transport vectors have rapidly increased the rate at which species are introduced into new environments (Ruiz *et al.* 2000; Hulme *et al.* 2008). The impact of non-indigenous species (NIS) on local communities can be difficult to quantify but often the effects are detrimental, ranging from reduced biodiversity (Wilcove *et al.* 1998), to dramatic habitat modification by ecosystem engineers (Crooks 2002; Neira *et al.* 2007). Consequently, it is important to understand the factors that enhance success at various stages of the invasion process (Kolar & Lodge 2001). One factor that is being increasingly recognized as pertinent for successful establishment is propagule pressure (Lonsdale 1999; Puth & Post 2005). Propagule pressure describes a measure of the number of individuals released into an area to which they are not indigenous (Carlton 1996). Therefore, the greater the supply of invasive propagules should increase the likelihood of establishment and, subsequently, invasion success (Lockwood, Cassey & Blackburn 2005). Many NIS are introduced into anthropogenically

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disturbed environments. Within these environments, disturbance may reduce the propagule pressure necessary for successful invasion by freeing resources (Davis, Grime & Thompson 2000; Lockwood, Cassey & Blackburn 2005). A more novel prediction is that anthropogenic disturbance will act directly to change the parameters of propagule pressure and therefore facilitate species invasion.

A particularly detrimental form of disturbance is contamination from anthropogenic sources (Foster & Charlesworth 1996). Pollutants such as metals, pesticides and oils can have profound impacts on communities, mostly by reducing species richness (Johnston & Roberts 2009; Brittain *et al.* 2010). Interestingly, tolerance to toxicants has also been observed, often in pest species for whom the toxicant was initially intended (Scarabel, Varotto & Sattin 2007). The prevalence of NIS in polluted environments (Dafforn, Glasby & Johnston 2009) suggests two things; first, contaminants themselves may be facilitating species introductions, through direct disturbance and the removal of native biomass (Crooks, Chang & Ruiz 2011). Second, because of a prior history of exposure and selection, certain NIS may be more tolerant to contaminants than native species within the receiving communities (Piola & Johnston 2008a, 2009). Tolerance to anthropogenic contaminants has evolved rapidly in numerous species, particularly after site-specific pollution results in a population being exposed to intense selection for multiple generations (Macnair 1987; Medina, Correa & Barata 2007).

Marine and estuarine environments in particular are very vulnerable to an increasingly widespread and toxic form of contamination: metal pollution (Hall, Scott & Killen 1998). Entering the system through numerous sources including the use of antifouling biocides (Weis & Weis 1996), sewage discharge and industrial effluent (Hall, Scott & Killen 1998), concentrations of metals typically reach their highest in areas of urban development such as harbours, ports and marinas. These habitats are also the most vulnerable to species introductions, being focal points for recreational and commercial shipping activities (Floerl *et al.* 2009). Boat hulls are a major vector for marine NIS (Clarke Murray, Pakhomov & Therriault 2011; Sylvester *et al.* 2011) and are often coated with copper-based antifoulant paints (Floerl & Inglis 2005); therefore, recruitment to and survival on hulls imply selection for copper tolerance in the fouling organisms being transported (Piola, Dafforn & Johnston 2009). This, coupled with increased propagule pressure from repeated introductions (Carlton & Geller 1993) and a recipient community with a lowered invasion resistance because of anthropogenic disturbance [e.g. by increasing resource availability (Davis, Grime & Thompson 2000)], may explain the increased presence of NIS in environments contaminated by metals. For example, a number of marine NIS have been found to have a positive affiliation with metal contamination, an affiliation that may enhance further range expansion (Dafforn, Glasby & Johnston 2009).

With propagule pressure increasingly recognized as a pertinent factor in the establishment and spread of NIS (Lockwood, Cassey & Blackburn 2005), it is important to understand how this component of the invasion process is affected by metal pollution. Recruitment is considered a key step in the introduction of a species, with increased invasion success linked to increased propagule pressure and disturbance (Clark & Johnston 2009; Crooks, Chang & Ruiz 2011). This study aimed to determine whether copper affects the recruitment process of an invasive species, the encrusting bryozoan *Watersipora subtorquata* (d'Orbigny 1852). *Watersipora subtorquata* is highly tolerant of copper (Piola & Johnston 2006), capable of recruiting to surfaces coated in antifoulant paint where it creates a less toxic secondary surface for other fouling organisms to settle on (Floerl, Pool & Inglis 2004). By providing a non-toxic refuge, *W. subtorquata* has been suggested to be capable of causing an 'invasion meltdown' by facilitating the transfer of other hull-fouling sessile invertebrates beyond their natural distribution (Floerl, Pool & Inglis 2004; although see Simberloff (2006)). This bryozoan has a cosmopolitan distribution; its native range is uncertain but thought to be in the Caribbean (Mackie, Keough & Christidis 2006). *Watersipora subtorquata* shares numerous similarities with many fouling NIS, for example, method of introduction, comparable physiological traits with other colonial organisms (such as brooded offspring and vegetative growth in ascidians) and a global distribution. Therefore, *W. subtorquata* is an appropriate organism for examining how a common metal pollutant, copper, affects the recruitment patterns of an invasive species. We did this by investigating how copper environments influence larval settlement, post-settlement survival and morphological characteristics of *W. subtorquata*. We found that high copper contamination strongly influenced recruitment patterns and morphological traits, with implications for continued range expansion by human vectors.

Materials and methods

To investigate the effect of copper on recruitment processes, we designed this experiment to mimic the settlement surface provided by a vessel's hull recently painted with copper-based antifouling biocide. Incomplete coverage of antifouling paint is common, because of the docking procedures preventing access to the entire hull, and creates a scenario which has been linked to the spread of invasive species (Piola & Johnston 2008b). It also simulates surfaces provided by permanent structures within urbanized estuaries which have been coated with biocides. Antifoulant biocides can leach dissolved copper at an initial rate of approx 25–65 μg cm^{-2} day^{-1} (Valkirs *et al.* 2003), resulting in concentrations that are lethal to many invertebrates and inhibit recruitment (Hall, Scott & Killen 1998).

We deployed settlement plates at one site, Burraneer Marina in Port Hacking estuary (34°7′S, 151°10′E) south of Sydney in New South Wales, Australia, during January and February 2009. This site has a high abundance of *W. subtorquata* colonies growing on artificial substrate and is an open marina. Settlement panels were hung horizontally at 1 m below mean low tide level with the settlement surface facing down, randomly located in shaded areas. Panels were treated as a replicate, either control or copper, to prevent any contamination between treatments with all panels hung a minimum of 2 m apart. For ease of experimental manipulation, each panel had four 11 by 11 cm Perspex settlement plates attached that had been roughened to encourage settlement. For the copper treatment, settlement plates were painted with a 2 cm wide border of the commercially available copper-based antifoulant paint (International® Micron Extra) (International, London, UK), while on control panels, the settlement plates were untreated. This left an identical, unpainted, 9 by 9 cm area of substrate on each plate on which settlement was compared for both copper and control treatments (Fig. 1). There were five panels per treatment and the entire experiment was repeated a month later at the same location (subsequently called trial 1 and 2).

Watersipora subtorquata release larvae from early in the day usually upon exposure to light and the larvae begin to settle immediately upon release (Marshall & Keough 2003). Hence, we deployed plates late in the afternoon to effectively sample the following day's recruitment. After approximately 40 h, plates were pulled out of the water, photographed and all *W. subtorquata* recruits circled with a graphite pencil for later identification and to monitor survival. We surveyed the entire plate excluding the border and counted any recruits that settled there. Although recruitment of other species into either environment was minimal, we removed any non-*W. subtorquata* recruits by scraping before redeploying the plates for 1 week to remove interspecific competition for resources. After 1 week, the plates were removed from the water and all surviving recruits from the initial settlement period were counted and photographed live with a digital camera (Leica Model: DFC290) using a dissecting microscope. Settler size, or the first zooid formed as a result of successful metamorphosis (Piola & Johnston 2006), has previously been shown to be a good predictor of initial larval size (Marshall & Keough 2003). Settler size has also been shown to significantly influence survival and growth of recruits during the initial 3 weeks after settlement (Marshall & Keough 2004). This initial zooid, or ancestrula, is easily delineated in small colonies because of the distinctive pattern of colony formation. We measured ancestrula length, from the top of the operculum to the distal edge of the zooid (length mm), and overall colony size (area mm^2). These morphological traits, ancestrula length and overall colony size, were measured to the nearest μm using the program LEICA APPLICATION SUIT Version 3.6 (Leica Microsystems GmbH, Wetzlar, Germany), before converting to mm.

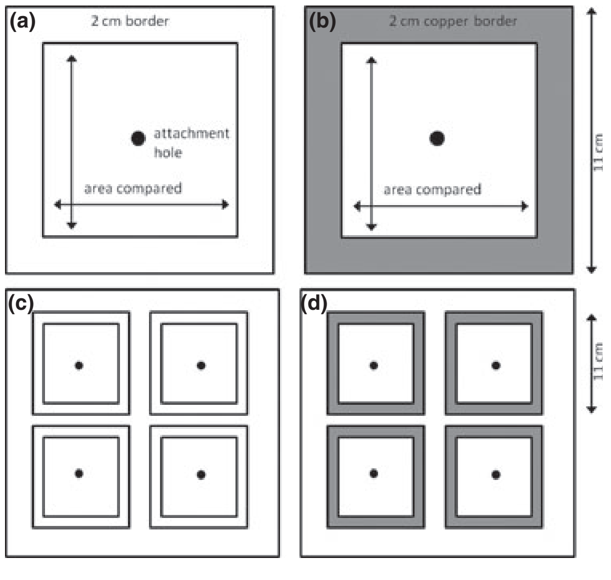

Fig. 1. Schematic of the settlement plates (a) control and (b) copper. Settlement plates in the copper environment were painted with a 2-cm border of copper-based antifouling paint, leaving an 9 by 9 cm unpainted area in the centre of the plate to be compared between treatments. Four settlement plates were attached to a panel, with (c) control and (d) copper settlement plates on separate panels. There were five panels of each treatment deployed for each trial.

STATISTICAL ANALYSIS

We compared initial settlement, final recruitment and post-settlement mortality between environments (copper and control) using a two-factor analysis of variance (trial: random, environment: fixed). Because there was no significant effect of the interaction between trial and environmental treatment ($P > 0{\cdot}2$) for each variable, the final model does not contain this term. Data were analysed as density per cm^2 for initial and final recruitment, and as a percentage for mortality using PERMANOVA in PRIMER v. 6 (PRIMER E Ltd., Plymouth, UK; Anderson 2001).

To test the effect of environment on morphology, we analysed colony size and ancestrula length using a two-factor mixed model multivariate analysis of variance (MANOVA; trial: random, environment: fixed). Ancestrula length and colony size were averaged for each panel, giving five replicates per treatment. We checked the data for homogeneity of variances and co-variances using Levene's and Box's test. Because there was no significant effect of the interaction between trial and environmental treatment ($P > 0{\cdot}2$), the final model does not contain this term. Data were analysed using SPSS v. 16 (SPSS Inc., Chicago, IL, USA).

Results

High numbers of *W. subtorquata* larvae recruited to the settlement plates, with densities ranging from 0·03 to 0·10 and 0·07 to 0·30 recruits per cm^2, on control and copper panels, respectively. Initial settlement was significantly greater on copper-painted panels ($F_{1,17} = 16{\cdot}78$, $P < 0{\cdot}001$; Fig. 2a). Post-settlement mortality was also significantly greater in the copper environment ($F_{1,17} = 35{\cdot}96$, $P < 0{\cdot}001$; Fig. 2c), with mortality reaching 49% on one panel compared with 0–17% on control panels. Despite the increased mortality, recruitment after 1 week was still significantly greater on copper-painted plates ($F_{1,17} = 9{\cdot}58$, $P = 0{\cdot}007$) with more than double the density of recruits (Fig. 2b). There was no difference between trials for initial settlement ($F_{1,17} = 1{\cdot}77$, $P = 0{\cdot}217$), final recruitment ($F_{1,17} = 1{\cdot}64$, $P = 0{\cdot}225$) or post-settlement mortality ($F_{1,1} = 0{\cdot}10$, $P = 0{\cdot}759$).

Of the *W. subtorquata* recruits that survived to 1 week, the majority exhibited growth with more than 96% having multiple fully formed feeding zooids. Overall, colonies appeared healthy with active lophophores, although there were a few mutated individuals in the copper environment. Recruit morphology was significantly impacted by the copper environment (Wilks' $\lambda = 0{\cdot}06$, $F_{2,16} = 129{\cdot}73$, $P < 0{\cdot}001$) for both traits. Colonies in the copper environment were significantly smaller in size ($F_{1,17} = 6{\cdot}75$, $P = 0{\cdot}019$) and had shorter ancestrula length ($F_{1,17} = 246{\cdot}49$, $P < 0{\cdot}001$; Fig. 3a,b) compared with colonies in the control environment. Morphology also differed significantly between trials (Wilks' $\lambda = 0{\cdot}58$, $F_{2,16} = 5{\cdot}78$, $P = 0{\cdot}013$), but this was attributable to ancestrula length ($F_{1,17} = 5{\cdot}24$, $P = 0{\cdot}035$) and not colony size ($F_{1,17} = 1{\cdot}61$, $P = 0{\cdot}221$; Fig. 3a,b). There was no significant interaction between trial and environmental treatment ($P > 0{\cdot}2$).

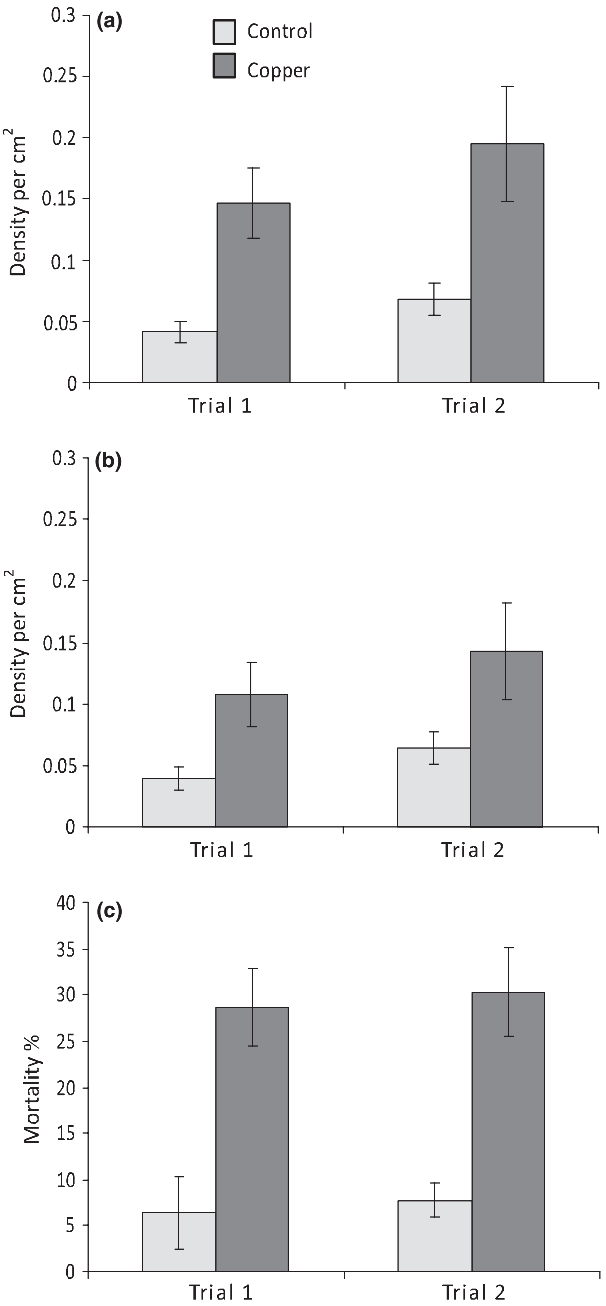

Fig. 2. Density per cm^2 of *Watersipora subtorquata* recruits (a) after 1 day of recruitment and (b) 1 week after initial recruitment, and (c) the mean mortality, on control and copper environments for each trial. Values are mean ± SE.

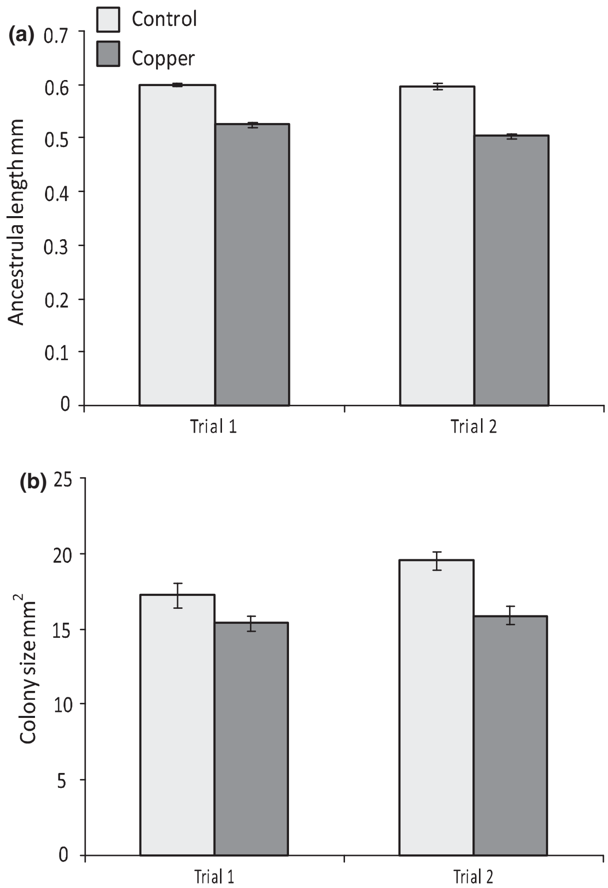

Fig. 3. The morphological traits: (a) ancestrula length (mm) and (b) colony size (mm^2), of recruits that survived to 1 week in control and copper environments at each trial. Values are mean ± SE.

Discussion

With the continued spread of NIS occurring at a global scale, it is important to understand whether managerial controls are inadvertently facilitating the invasion of contaminant tolerant species. In an unprecedented result, we found that a commonly used biocide, copper, significantly increased recruitment of a NIS, despite associated fitness costs and mortality. While previous research has found *W. subtorquata* to be capable of recruiting near or onto copper-based antifoulant-coated surfaces (Floerl, Pool & Inglis 2004), this study has shown a dramatic increase in recruitment to panels with copper. The positive affiliation we found between *W. subtorquata* and copper has consequences for two components of the invasion process. First, it is likely to promote the continued spread of the species as there is an increased likelihood of recruitment onto a vector surface, such as a ship hull. Secondly, it is likely to further facilitate recruitment into environments that are impacted by copper contamination, notably most urbanized harbours and ports. Therefore, copper contamination has the capacity to directly enhance propagule pressure by increasing the supply and retention of individuals into anthropogenically disturbed environments.

Recruitment is a fundamental demographic process that can strongly affect population dynamics (Minchinton & Scheibling 1991; Myers & Harms 2009). For many organisms, recruitment involves habitat choices by individuals using environmental cues to ascertain habitat quality, which influences short- and long-term success (Pawlik 1992; Stamps, Luttbeg & Krishnan 2009). Despite the toxicity of copper (Bryan 1971; Hall, Scott & Killen 1998), settlement was significantly greater in the copper environment, with densities reaching up to three times higher than in the control environment. These densities remained substantially greater, by more than double, even after greater post-settlement mortality in the copper environment. This is rather unusual as copper contamination has been shown to reduce recruitment for an array of marine invertebrate species (Johnston, Webb & Keough 2003), suggesting that *W. subtorquata* may be responding to copper as a settlement cue or that copper is inducing settlement. Furthermore, very few contaminants have been linked with an increase in population density through directly enhanced recruitment or population migration. Such a positive response is usually restricted to nutrient additions, for example, enhanced recruitment of oyster and barnacle larvae (Minchinton & McKenzie 2008), which has been attributed to the contaminant either enhancing habitat quality or mimicking cues that signify quality habitat.

Preference for 'poor' quality habitat is usually attributed to misleading environmental cues creating an ecological trap (Battin 2004). However, given the increased recruitment in the copper environment despite the availability of abundant clean alternate habitat, it seems plausible that *W. subtorquata* larvae may be using copper pollution as an environmental cue for settlement, particularly as this species has been shown to exhibit settlement preferences regarding substrate types (Marshall & Keough 2003). In addition, hull fouling has been identified as an important vector for this cosmopolitan invasive species (Floerl, Pool & Inglis 2004), which suggests this positive affiliation is a result of historical exposure and selection. This affiliation may be a direct influence of copper or an indirect effect. For example, changes to bacterial biofilm composition caused by copper contamination can reduce recruitment or biofilm inductiveness (Bao *et al.* 2010), a potentially important effect considering biofilms are recognized as an important settlement cue for many invertebrate larvae (Pawlik 1992).

Early life-history stages can be particularly susceptible to contaminants because they are periods of rapid growth and metamorphosis. In conjunction with greater recruitment, mortality was also significantly greater in the copper environment, with an average of almost 30% of the recruits dying after settlement. This is unsurprising considering the toxic effect of copper on many organisms (Hall, Scott & Killen 1998) and its specific use as a biocide in the marine environment (Piola, Dafforn & Johnston 2009). Instead, the combination of high recruitment and high mortality suggests that copper is exerting strong selection on each cohort of recruits after settlement. When a contaminant has a negative impact on the recruitment process, it can cause rapid shifts in the genotypic composition of a population, selecting for more tolerant individuals (Eranen 2008). This has been seen in both the aquatic and terrestrial environment, where populations of acutely exposed organisms have rapidly evolved tolerance [e.g. aquatic oligochaete (Klerks & Levinton 1989), wolf spider (Hendrickx, Maelfait & Lens 2008) and mountain birch (Eranen 2008)]. Additionally, the high mortality observed here suggests that copper tolerance is not consistent throughout this introduced population, indicating a likely opportunity for further selection of this trait. This selection for tolerant genotypes might enhance the capacity for some genotypes to survive in highly contaminated environments, and as this would most likely include the hull of a vessel, it may augment this species invasion potential.

Morphologically, recruits differed dramatically between the control and copper environments, having shorter ancestrula and smaller overall colony size in the contaminated conditions. These differences indicate that the recruits that are capable of surviving in a copper environment may be suffering short- and long-term fitness effects (Ng & Keough 2003). Overall, morphological differences between the two environments are not surprising, assuming the high mortality levels have selected for tolerant genotypes (Eranen 2008). Comparisons between populations with and without a history of exposure have found physiological differences such as reduced size and changes in reproductive strategy (Shirley & Sibly 1999; Hendrickx, Maelfait & Lens 2008), which are attributable to reallocation of resources to deal with the metabolic costs of tolerance (Shirley & Sibly 1999). Therefore, reduction in size may indicate a trade-off between growth and tolerance, which would then lead to longer-term impacts on reproduction and fitness.

Offspring size is strongly linked with individual fitness, with general trends predicting greater survival and fitness in larger offspring for a variety of organisms (Smith & Fretwell 1974). For organisms that have a larval stage, recruit size after metamorphosis is a pertinent measure, as it represents larval history and size as well as future fitness potential (Pechenik, Wendt & Jarrett 1998). With ancestrula length as a representative measure of initial recruit size, the disparity in lengths between the two environments indicates that copper is affecting the recruitment process beyond simple mortality. The most likely point of greatest impact would be during metamorphosis, with copper disrupting the process and changing ancestrula morphology, which is further indicated by the presence of malformed recruits. When not immediately lethal, contaminants can delay or facilitate metamorphosis in many organisms [e.g. amphibians (Gross, Chen & Karasov 2007)]. The differences in ancestrula length between trials would most likely reflect variation in environmental conditions of the parent colony during brooding period, offspring size being a result of parental environment and genotype (Bernardo 1996), resulting in variation between larval/recruit sizes over time.

These recruitment patterns provide insight into how this species has become such a successful and cosmopolitan invader (Mackie, Keough & Christidis 2006), especially if responding to metal pollution as a settlement cue. And while these recruitment patterns initially appear to be a maladaptive habitat choice, a consequence of choosing to recruit to a copper contaminated environment is an intense increase in propagule pressure if individuals survive to reproduce. It also indicates that the spread of this species will not be curbed through the use of copper-based antifoulant paints, instead increasing use may actually be facilitating invasion of copper-tolerant genotypes. Therefore, the heavy reliance on copper-based antifouling practices needs to be reconsidered and more sophisticated management methods developed, potentially using rotating biocidal chemicals or non-toxic biocides (Qian, Xu & Fusetani 2010). Metal contamination is an issue in urbanized estuaries (Birch & Taylor 1999), in part because of current antifouling practices that rely heavily on copper-based biocides (I.M.O. 2001; Srinivasan & Swain 2007). Despite high mortality and fitness costs, *W. subtorquata* larvae recruited considerably more into the copper environment, where numerous individuals were capable of surviving beyond the initial recruitment process. Hence, we observed an anthropogenic disturbance directly increasing the likelihood of establishment success of a NIS. Managing the introduction of NIS therefore requires metal contamination to be reduced in areas where inoculation events are more frequent (Carlton & Geller 1993; Floerl & Inglis 2005). Reducing the use of copper-based biocides on vectors and remediation of recipient environments, such as harbours ports and marinas, could substantially reduce the transfer of metal tolerant organisms and their associated epibiota.

Considering that other sessile marine invertebrates already show a positive affiliation with metal pollution (Dafforn, Glasby & Johnston 2009; Piola, Dafforn & Johnston 2009; Crooks, Chang & Ruiz 2011), there is the possibility that this relationship has already evolved and/or has the evolutionary potential to evolve further in other invasive species. We suggest that the trait of pollution tolerance may be a useful criterion for identifying whether a species is non-indigenous, when considered in conjunction with criteria listed by Chapman & Carlton (1991). These already include an association with human transport mechanisms and artificial habitat (Chapman & Carlton 1991), but in scenarios where this may be difficult to discern, identifying a positive affiliation for metal-polluted environments would convey equivalent information.

With the spread of NIS increasing on a global scale, there is a need to consider how anthropogenic activities, such as pollution, can influence the invasion process. In this study, we have identified the novel effect of how a common anthropogenic contaminant can directly facilitate species invasion by changing the parameters of propagule pressure. Therefore, when attempting to curtail the spread of NIS, understanding this interaction will enhance the effectiveness of managerial controls for reducing both spread and establishment success.

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