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Comparing differential tolerance of native and non-indigenous marine species to metal pollution using novel assay techniques

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Greater tolerance to pollutants in marine NIS may increase the risk of invasion in port and harbours worldwide by providing a competitive advantage over native taxa.

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ABSTRACT

Recent research suggests anthropogenic disturbance may disproportionately advantage non-indigenous species (NIS), aiding their establishment within impacted environments. This study used novel laboratory- and field-based toxicity testing to determine whether non-indigenous and native bryozoans (common within marine epibenthic communities worldwide) displayed differential tolerance to the common marine pollutant copper (Cu). In laboratory assays on adult colonies, NIS showed remarkable tolerance to Cu, with strong post-exposure recovery and growth. In contrast, native species displayed negative growth and reduced feeding efficiency across most exposure levels. Field transplant experiments supported laboratory findings, with NIS growing faster under Cu conditions. In field-based larval assays, NIS showed strong recruitment and growth in the presence of Cu relative to the native species. We suggest that strong selective pressures exerted by the toxic antifouling paints used on transport vectors (vessels), combined with metal contamination in estuarine environments, may result in metal tolerant NIS advantaged by anthropogenically modified selection regimes.

1. Introduction

The introduction and spread of non-indigenous species (NIS) is considered one of the foremost threats to the ecology of terrestrial, freshwater and marine systems (Groves and Burdon, 1986; Carlton, 1989; Czech et al., 1997; Ruiz et al., 1997; Wilcove et al., 1998; Dextrase and Mandrak, 2006; Dudgeon et al., 2006). Many species introductions have modified ecosystems through reductions in native diversity, increased predation pressure, habitat alteration and competition for available resources (Mack et al., 2000). Over the last 50 years, the observed rates of invasion in coastal systems have risen considerably across many varied habitat types and taxonomic groups (Cohen and Carlton, 1998; Ruiz et al., 2000; Occhipinti-Ambrogi and Savini, 2003; Hewitt et al., 2004; Grosholz, 2005). Experimental research to investigate potential causes of invasibility and to explain the pattern of increasing invasion success has lagged behind theoretical and observational studies.

Links between the invasibility of a system and the level of disturbance, or degradation, experienced by that system have long been recognised (Elton, 1958; Fox and Fox, 1986). Both natural and anthropogenic disturbances can affect the biotic and abiotic characteristics of natural systems –influencing a community's ability to resist invasion (Ruiz et al., 2000). For example in marine systems, newly created bare space resulting from physical disturbance can increase the recruitment success of invasive sessile invertebrate taxa within hard substrate assemblages (Clark and Johnston, 2005; Altman and Whitlatch, 2007). Few studies however, have tried to discern specific responses of native and NIS to anthropogenic impacts and disturbances (but see Jewett et al., 2005; Tyrrell and Byers, 2007).

Metal pollution is a common anthropogenic disturbance within estuarine environments worldwide. Copper (Cu) in particular, is one of the most commonly occurring metal pollutants, originating from a wide range of sources including antifouling coatings (Warnken et al., 2004), industrial waste (Hall Jr. et al., 1998), urban runoff (Pitt, 2002), sewage discharge (Scanes, 1996) and wood preservatives (Weis and Weis, 2002). Cu has been shown to have detrimental effects on the health and success of many species (Hall Jr. et al., 1998), and has been shown to play a key role in the transportation and establishment of NIS (Floerl et al., 2004; Piola and Johnston, 2006b).

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Many introduced marine species arrive to new areas as fouling organisms attached to the hulls of ships (Gollasch, 2002; Hewitt, 2002; Minchin and Gollasch, 2003). This is particularly true of sessile invertebrate taxa such as bryozoans, ascidians, hydroids, serpulid polychaetes and barnacles (Hewitt et al., 2004). Interestingly, such hull fouling assemblages often establish on vessel hulls despite the presence antifouling biocides, such as copper-based paints (Floerl et al., 2004). As such, hull fouling is a vector for species transport that is highly selective for metal tolerant organisms. It becomes important therefore, to determine whether these metal tolerant NIS benefit from a competitive advantage over native species at recipient locations (such as harbours and ports) that are themselves subject to transient or persistent metal-pollution events.

Despite evidence of links between metal pollution and the occurrence of introduced species, few studies have examined the possible relationship between the two (but see Piola and Johnston, 2008a). This situation is somewhat surprising if we consider that harbours and estuaries, the primary recipient locations of NIS in marine systems (Ruiz et al., 1997), also rank amongst the most contaminated environments worldwide (Hall Jr. et al., 1998). Are the observed increases of NIS in harbours and estuaries worldwide simply a manifestation of increased inoculation rates (through a range of vectors such as shipping or aquaculture), or are growing numbers of invasive organisms better adapted to take advantage of increasingly chemically disturbed environments? By studying the differential effects that specific types of toxic waste have on non-indigenous and native taxa, we may better understand possible links between anthropogenic disturbance and the establishment and spread of NIS.

Laboratory assays examining the effects of toxicants on model "indicator" species continue to provide a mainstay for aquatic environmental toxicologists (Chapman, 2002). Such tests tend to focus on larval toxicity, since larvae are generally recognised as the invertebrate life-stage most sensitive to toxicants (Connor, 1972; Calabrese et al., 1973; McKim, 1977). Common end-points for such laboratory-based dose–response studies include the larval attachment success and/or post-settlement metamorphic success (Wisely, 1958; Ng and Keough, 2003; Bellas et al., 2004; Piola and Johnston, 2006a). While these types of experiments are undoubtedly useful (e.g. for the rapid assessment of large suites of potentially hazardous substances), they remain simple and are conducted under highly controlled conditions that do not reflect real-world environments. Additionally, such tests often fail to consider other important factors including; the effect of toxicants on adult life-stages, and the enhanced real-world effects of toxicants in combination with additional stressors such as poor water quality, habitat loss, natural disturbance events and nutrient enrichment (Preston and Shackelford, 2002; Goldman Martone and Wasson, 2008).

Bryozoans form an abundant component of most epibenthic communities (Gordon and Mawatari, 1992), and are common fouling organisms on the hulls of ocean-going vessels (Gordon and Mawatari, 1992; Minchin and Gollasch, 2003; Floerl et al., 2004). This study aims to compare of the tolerance of native and NIS bryozoan species to the common heavy metal pollutant Cu, under laboratory and real-world field conditions. Using a series of novel testing techniques we compare the Cu sensitivity of both larvae and adults of four well-recognised cosmopolitan invasive bryozoans (Gordon and Mawatari, 1992), *Watersipora subtorquata*, *Bugula neritina*, *Schizoporella errata* and *Tricellaria inopinata* (also listed as *Tricellaria occidentalis*), with that of two native bryozoans, *Celleporaria nodulosa* and *Fenestrulina mutabilis*. *W. subtorquata*, *B. neritina*, *S. errata* and *T. inopinata* are dominant species in south-east Australian fouling communities, and all listed as national priority pest species within Australian waters, with invasion potentials ranging from medium-low to high (Hayes et al., 2004).

2. Methods

2.1. Adult laboratory and field assays

2.1.1. Copper treatments

In all experiments, analytical grade copper II chloride hydrous ($CuCl_2 \cdot 2H_2O$) was used as the reference toxicant. A 1000 μg l^{-1} Cu solution was prepared from this stock solutions each day and diluted in order to obtain all experimental treatment solutions of 10, 25, 50 and 100 μg l^{-1} Cu. Filtered seawater collected from the field site was used as the dilution medium. All equipment was acid washed in 5% nitric acid for a minimum of 24 h and rinsed in Milli-Q® filtered water prior to use.

2.1.2. Laboratory toxicity experiments

The health and survival of adult bryozoan colonies was determined during 18 d laboratory toxicity assays. Colonies of *W. subtorquata*, *S. errata*, *C. nodulosa* and *F. mutabilis* were collected by divers from the relatively unpolluted area of Kurnell Pier, in Botany Bay, NSW Australia. Botany Bay is a large, well-flushed marine-dominated estuary, which in spite of its urbanisation has retained many natural areas, including aquatic reserves (Pollard and Pethebridge, 2002). The experimental site used in this study is close to the mouth of the bay and is regularly flushed with oceanic water. In the laboratory multiple colonies of each species were dissected into small fragments of approximately 0.25 cm^2 using a scalpel, with particular attention being made to ensure no experimental colony fragment included an active growing edge of the original colony. Fragments were placed into individual 70 ml plastic containers with filtered seawater and allowed to recover for 7 d prior to the commencement of the experiment. Following recovery, bryozoan colonies were haphazardly assigned to one of five experimental Cu treatments comprising control seawater, 10, 25, 50 or 100 μg Cu l^{-1} (n = 10–12 replicates per concentration). Replication was slightly unbalanced due to variability in the availability of bryozoan colonies. Concentrations of 10, 25 and 50 μg l^{-1} Cu represent relevant values that do exist in polluted aquatic environments (Moran and Grant, 1989; Stauber et al., 2000; Schiff et al., 2004), while the 100 μg l^{-1} Cu concentration was included to gauge maximum tolerance limits. For the duration of the experiment, the microalgae *Isochrysis galbana* (clone T.Iso) was offered daily as food to the colonies as a component of the treatment solutions at 10^5 cells ml^{-1}. All Cu treatment/T.Iso solutions were replaced every 24 h with fresh solutions. Exposure to Cu was maintained for a period of 9 d, after which colonies were transferred to individual containers with filtered seawater for a further 9 d. International shipping voyages usually take 6–10 d to reach Australia and hence bryozoans being transported on ship hulls may be exposed to copper-based antifouling paints for approximately the period used in our tests. Any colony surviving 9 d of exposure to 100 μg l^{-1} Cu is very likely capable of being transported on a freshly painted ship hull.

During the exposure and recovery periods, various indicators of colony health were recorded for comparison among NIS and native species, including changes in biomass of the colony, growth and feeding efficiency. Changes to colony biomass over the entire 18 d period of the experiment were measured by weighting individual colonies at day 0, and day 18 using an electronic balance. Growth of colonies was determined by measuring changes in surface area (SA) using digital photographs and Image Pro Express Version 4.0.1 (Media Cybernetics Inc., Bethesda, MD, USA) image analysis software. The SA of all experimental colonies was recorded at three day intervals for the duration of the experiment. Feeding efficacy of the colonies was measured by counting the number of T.Iso cells remaining in experimental treatment solutions following a 24 h feeding period. Every three days, five colonies were randomly selected from each Cu-treatment group and the number of T.Iso cells remaining at the end of the 24 h feeding period was calculated using a haemocytometer (AO Spencer Bright Line No. 730239). The fewer T.Iso cells remaining in solution after 24 h, the greater the feeding efficiency of the colony.

Experiments were conducted in a controlled temperature room maintained at 20.0 ± 0.1 °C. Water temperature, salinity, pH and dissolved oxygen were recorded daily for all new (0 h) and old (24 h) Cu-treatment solutions using a YSI 556 MPS® (Yellow Springs, OH, USA) water quality meter. Water samples were collected from randomly selected treatment containers throughout the experiment to determine total Cu after 0 h (n = 2 for each species), total Cu after 24 h (n = 1 for each species) and dissolved Cu remaining in solution after 24 h (n = 1 for each species). These samples were analysed at the Australian Government National Measurement Institute in Sydney, Australia, using ICP-AES (detection limit of 5 μg l^{-1}).

A nematode infection was observed in the *F. mutabilis* colonies nine days into the experiment (affecting 90% of controls, 30% of 10 μg l^{-1} Cu treatments and 10% of 25 μg l^{-1} Cu treatments). Though nematode densities were low (average 5 per colony) they did appear to affect colony biomass and growth in some control colonies. Interestingly, Cu appeared to prevent nematode infection. Only a small number of nematodes were observed in 10 and 25 μg l^{-1} Cu treatment (3 and 1 respectively), with very low densities of worms (~1 per colony). No nematodes were observed in 50 and 100 μg l^{-1} Cu treatments. Given that the majority of *F. mutabilis* experimental treatments were unaffected by nematodes (i.e. 10, 25, 50 and 100 μg l^{-1} Cu) the experiment was continued until its conclusion at 18 d.

2.1.3. Field toxicity experiments

A field-based adult transplant experiment was conducted to assess the health and survival of adult bryozoan colonies exposed to Cu under realistic conditions. The