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Contaminant cocktails: Interactive effects of fertiliser and copper paint on marine invertebrate recruitment and mortality



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ABSTRACT

Understanding interactive effects of contaminants is critical to predict how human activities change ecosystem structure and function. We examined independent and interactive effects of two contaminants (fertiliser and copper paint) on the recruitment, mortality, and total abundance of developing invertebrate communities in the field, 2, 4, 6, and 8 weeks after substrate submersion. Contaminants affected community structure differently, and produced an intermediate community in combination. Fertiliser increased recruitment and decreased mortality of active filter feeders (ascidians and barnacles), while copper paint decreased recruitment and increased mortality of some taxa. Contaminants applied together affected some taxa (e.g. Didemnid ascidians) antagonistically, as fertiliser mitigated adverse effects of copper paint. Recruitment of active filter feeders appears to be indicative of nutrient enrichment, and their increased abundance may reduce elevated nutrients in modified waterways. This study demonstrates the need to consider both independent and interactive effects of contaminants on marine communities in the field.

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1. Introduction

Human activities impact on all marine systems, with the highest cumulative impacts occurring in areas subject to both land and ocean based anthropogenic drivers (Halpern et al., 2008). Exposure of marine ecosystems to multiple chemical contaminants is increasingly common, and the interactive effects of these contaminant 'cocktails' can influence the structure and function of ecosystems (Vinebrooke et al., 2004). Effects of contaminants have traditionally been examined individually, although investigations of the effects of simultaneous exposures are growing in number (reviewed by Crain et al., 2008). While establishing definitive causal relationships between stressors and their effects in natural environments is complicated (reviewed by Adams, 2005), understanding these relationships and how they influence marine systems is crucial to the effective management and rehabilitation of estuaries.

Assessing contaminant impacts in field environments is complex due to natural variability and the diversity of effects elicited by a range of stressors (Folt et al., 1999). In general terms, the effects of contaminant stressors can be additive (combined effects equal to the sum of effects of individual stressors), synergistic (greater effect) or antagonistic (decreased effect; Crain et al., 2008; Fitch and Crowe, 2012; Folt et al., 1999). It has been suggested that interactive effects are not predictable from knowledge of the impacts of individual stressors (Paine et al., 1998) and that such interactions could alter community or system

* Corresponding author. E-mail address: j.lawes@unsw.edu.au (J.C. Lawes). structure (Darling and Côté, 2008). Experimental manipulations of stressors in the field are therefore needed to explore independent and interactive effects (Halpern et al., 2008).

Estuaries are widely regarded as one of the most impacted of all marine ecosystems (Halpern et al., 2008) and the declining quality of many estuarine habitats is well recognised (Lotze et al., 2006). Estuaries are often subject to high levels of industrial and recreational activity that impose multiple stressors, including exposure to a wide range of chemicals. Two of the most commonly reported stressors within estuaries are excess nutrients and metals. Both stressors are well studied individually and can have significant consequences for the structure and function of estuarine ecosystems (Carnell and Keough, 2014; Johnston and Roberts, 2009), yet experimental studies of their interactive effects have, until recently, been relatively rare (but see Atalah and Crowe, 2012).

Major sources of nutrient pollution in coastal areas include agriculture, urban run-off, and nitrogen deposition from fossil fuel combustion (Selman and Greenhalgh, 2009). Nutrient enrichment can occur via the air, surface water, or groundwater, and predominantly results in elevated levels and altered stoichiometry of nutrient-rich inorganic elements, such as nitrogen and phosphorous. The combined effects of these elements can alter ecosystem function by enhancing primary production and subsequent herbivory rates (Touchette and Burkholder, 2000), or by modifying settlement cues for invertebrates (Sawall et al., 2012). High concentrations of excess nutrients can also result in eutrophication and hypoxia (Howarth, 2008), causing mortality. By these mechanisms, changes in nutrient levels and composition (i.e. stoichiometry) can

dramatically alter the abundance and diversity of marine invertebrates, as demonstrated in a variety of habitats including intertidal reefs (Atalah and Crowe, 2010; Atalah and Crowe, 2012), mangroves (Minchinton and McKenzie, 2008), and benthic macrofauna (Fitch and Crowe, 2012). Nutrients have important consequences for the demographic and ecological processes that regulate marine population dynamics (Minchinton and McKenzie, 2008), but their interaction with other stressors is still being explored (e.g. Atalah and Crowe, 2010; Fitch and Crowe, 2012; Jara et al., 2006; O'Gorman et al., 2012; Sugden et al., 2008).

Metals are the most common and widespread group of toxic contaminants found in estuarine systems. They are used in a number of activities and can persist in the marine environment, often bound to seafloor sediments (Hedge et al., 2009). Copper, in particular, is a common toxicant in the marine environment that has demonstrated effects on macrofouling invertebrate assemblages (Piola and Johnston, 2008a), particularly as the main toxicant in antifouling paint. Industrial waste (Hall et al., 2009), urban runoff, sewage discharge (Scanes, 1996), and antifouling biocides (Dafforn et al., 2011; Johnston et al., 2011) are common contributors to copper pollution in estuaries. Exposure to copper has been demonstrated to reduce the abundance, growth and reproductive success of marine organisms (Hall et al., 2009; Xie et al., 2005), and alter the structure of marine assemblages (e.g. Johnston et al., 2002).

Metals are generally harmful to marine invertebrates, while nutrient enrichment can encourage primary production and food availability. It is therefore possible that these two stressors may interact antagonistically, whereby nutrient enrichment could mitigate some of the toxic effects of copper (Crain et al., 2008). For example, the bioavailability of metals may decrease after binding with high concentrations of organic matter (McCarthy and Bartell 1988), or competition between intracellular nutrients and metals may reduce, or mitigate, toxic effects (Twiss and Nalewajko, 1992; Wängberg and Blanck, 1990). The chemistry of toxicants, in terms of concentration and chemical species (i.e. bonding and debonding actions) may also affect how their interaction manifests within marine environments (Sunda and Huntsman, 1998) and modulate the toxicological responses of biota (Serra et al., 2010). Although the importance and complexity of these mechanisms are acknowledged, this study did not attempt to determine the mechanism by which interactive effects occur. Instead, we focus on detecting evidence of such effects on macrofouling community in the

Hard-substrate macrofouling invertebrate communities are often used as model communities to detect human impacts in estuaries as they constitute a significant component of biodiversity within these systems and are forced to withstand pollution events due to their sessile nature (Knott et al., 2009). Macrofouler abundance is predominantly a function of recruitment and mortality within a community (Gaines and Roughgarden, 1985; Minchinton and McKenzie, 2008) and the successful recruitment of larvae (and their survival) provides a sensitive test of the effects of contaminants (King et al., 2006). Here we use the term 'new recruit' to refer to any organism censused for the first time within a community; in the case of sessile invertebrates these are generally recently settled individuals (see Minchinton and Scheibling, 1993). The ongoing development of the assemblage (measured by recruitment and mortality) enables assessment of indirect effects through time-mediated species interactions (Johnston and Keough, 2003). This study investigated the independent and interactive effects of exposure to fertiliser and antifouling paint on sessile marine community development. We hypothesised that locally elevated nutrient and copper levels will have opposing effects on community structure via changes in recruitment and mortality through time. A further prediction is that there would be an antagonistic effect of these two stressors, with elevated nutrients mitigating the toxic effects of copper exposure through increased recruitment and decreased mortality within combined treatments.

2. Materials and methods

2.1. Study site and experimental design

To investigate the independent and interactive effects of a copper antifouling paint and a fertiliser on the development of sessile invertebrate assemblages, a field experiment was conducted over 8 weeks at the Sydney Institute of Marine Science (SIMS) wharf, Chowder Bay, Australia. This site was selected as it is considered relatively uncontaminated within Sydney Harbour (Dafforn et al., 2012).

The design constituted three orthogonal factors: copper paint (copper or no-copper), fertiliser (elevated or ambient nutrients), and time (2, 4, 6, and 8 weeks). Each treatment was applied to five treatment backing panel replicates ($400 \times 400 \text{ mm}$) to which three acrylic plastic recruitment plates (110 \times 110 mm) were attached. The front side of each backing panel was covered by a $400 \times 400 \times 100$ mm plastic mesh (15 × 15 mm mesh size; Boddingtons™) cage that allowed sessile invertebrate recruitment and growth under conditions of reduced fish predation. At each census period, the mesh cage was cleaned of algae and sediment to maintain water flow throughout the experiment. Cage controls and uncaged treatments were not included since all treatments were caged. Effects of predation were beyond the scope of this study. Each panel was attached vertically to a rope that was weighted with a brick, and assigned to a randomly allocated position underneath Chowder Bay wharf with a minimum distance of 1 m between each treatment panel. This was considered adequate to ensure replicate independence in terms of treatment due to tidal flushing with oceanic water from nearby heads of Sydney Harbour, and was confirmed by ambient water analysis (see the Water contaminant analyses section below). The panels were located 1–1.5 m below low tide mark to guarantee all panels were fully submerged.

2.2. Fertiliser treatments

Each perspex plate was bordered by 4 × 10 mL perforated (40 × 1 mm diameter holes) centrifuge tubes, herein referred to as 'nutrient tubes'. Nutrients tubes contained Osmocote™, a common fertiliser that has unrestricted use for food and animal feed crops. These perforations were designed to allow water flow through the tube, facilitating nutrient release (see Minchinton and McKenzie, 2008). As a procedural control, empty nutrient tubes were attached to treatments not containing nutrients to control for possible flow changes caused by the tubes located near the recruitment plates. Each nutrient tube contained 10 g of Osmocote™ slow-release fertiliser (landscape formula) with a N:P:K ratio of 17.7:2:5.8. This included 17.7% N (2.5% ammonium N, 13.6% urea N, 1.7% nitrate N), 2% P (1.6% water soluble P, 0.4% citrate soluble P), and 5.8% K (as a sulphate), as well as sulphur (8.3%), magnesium (2.2%), a non-ionic wetting agent (3.3%) and a vegetable oil-based coating (2.7%). Essential trace elements were incorporated as compounds (see MSDS, 2010; www.scottsaustralia.com.au) including boron, copper, manganese, iron, molybdenum, zinc, cadmium and lead (each were < 1% proportion of ingredients). Fertiliser levels were checked at each census point but did not require replacement throughout the duration of the experiment. This fertiliser was selected to simulate urban catchment run-off, which commonly augments nutrient levels within estuaries (Selman and Greenhalgh, 2009).

2.3. Copper antifouling treatments

Copper was introduced via copper-containing antifouling paint (Micron Extra, International PaintsTM; see MSDS, 2011; http://datasheets.international-coatings.com/msds/YBA944E1_GBR_ENG.pdf) comprising copper (I) oxide (25 < 50%), zinc oxide (10 < 25%), rosin (10 < 25%), xylene (2.5 < 10%), solvent naptha (2.5 < 10%), 1,2,4 < trimethylbenzene (2.5 < 10%), ethylbenzene (1 < 2.5%) and dichlofluanid (0 < 1%). Although there are many compounds within

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