Marine Pollution Bulletin xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Impacts of multiple stressors during the establishment of fouling assemblages

Silvia Saloni *, Tasman P. Crowe

School of Biology and Environmental Science, Science Centre West, University College of Dublin, Belfield, Dublin 4, Ireland

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Multiple stressors
Biodiversity
Ecosystem functioning
Recruitment
Copper
Salinity

ABSTRACT

Limited knowledge of the mechanisms through which multiple stressors affect communities and ecosystems limits capacity to predict their effects. Less clear is how stressors impact early colonization of newly available habitats due to scarcity of studies. The present study tested whether copper and freshwater input affect colonization of hard substrata independently or interactively and assessed differences in community respiration and total biomass among early stage assemblages which developed under different regimes of copper and freshwater input. While copper influenced effectively the colonization of individual species, freshwater effect was weak or null. Apart from a significant effect on total community composition, the interactive effect between stressors was weak and mainly driven by antagonistic interactions between copper and water flow. Total biomass and respiration of the community studied were not affected by stressors. These findings contradict the expectation that changes in community structure are likely to cause changes in functioning.

1. Introduction

Most marine ecosystems are affected by two or more anthropogenic stressors (Halpern et al., 2008). Most research into effects of stressors, however, has focused on effects of individual stressors (Crain et al., 2008; Darling and Côté, 2008). Improved understanding of the combined effects of multiple stressors on diversity and ecosystem functioning is essential to predict and buffer anthropogenic pressure on natural ecosystems (Vinebrooke et al., 2004). Multiple stressors often interact and their combined effect is therefore unpredictable from their individual effects (Folt et al., 1999). Stressors are considered to interact synergistically when their joint effect is greater than would be estimated from their individual effects, and antagonistically when their combined effect is less than expected based on their individual effects. Synergism and antagonism are detected as departures from a null model, which is usually additive or multiplicative. While the additive model assumes that stressors are not interacting if their combined effect can be calculated by summing their individual effects, the multiplicative model considers that two stressors are acting independently when their combined effect is equal to their product (Folt et al., 1999).

The effect of stressors also depends on several factors, such as time of exposure, intensity and variance of the stress regime (Benedetti-Cecchi et al., 2006), environmental conditions and tolerance of organisms under study and their interaction (DeAngelis, 1996; Johnston and Keough, 2002). Given this complexity, it is a hard task to predict how different stressors work together to affect biodiversity and ecosystem functioning and more research is needed (Crain et al., 2008; Darling and Côté, 2008). To date, there has been a tendency to focus on adult stages in mature assemblages and less is known about how stressors can disrupt early colonization of newly available habitats. The presence or the absence of a species in a stressed environment depends on the survivorship of its most sensitive life stage (Beaumont et al., 1987; Ogburn et al., 2012). Understanding which life stages (in terms of ontogeny and succession) are the most vulnerable to anthropogenic or natural stressors would help management of human activities to reduce their impact on biota.

Coastal environments are among the most threatened ecosystems and are quite likely to suffer increased rates of local extinction in the coming years (Crowe et al., 2000; Halpern et al., 2008). Heavy metals, mainly derived from industrial, urban, agricultural and mining wastes, represent a major threat to coastal ecosystems (Mayer Pinto et al., 2010). Copper is among the heavy metals most widely used in different human activities (e.g. antifouling paints, wood preservatives). Hydrated copper ions have been shown to be very toxic for marine organisms (Johnston et al., 2002), potentially interfering with photosynthetic activity and nitrogen uptake of macroalgae (Gledhill et al., 1999; Eklund and Kautsky, 2003) and obstructing the metabolic and respiratory activity of marine invertebrates (Johnston and Keough, 2002). The

* Corresponding author. Tel.: +353 (1) 716 2829.
E-mail address: salonisilvia@gmail.com (S. Saloni).

http://dx.doi.org/10.1016/j.marpolbul.2014.12.003
0025-326X/

biodiversity of marine coasts is also heavily threatened by climate change (Johnston and Roberts, 2009; Richardson et al., 2012), especially by extreme events (i.e. storms and flooding) and by coastal inundation and erosion caused by increased sea level (Nicholls et al., 2007). Extreme rainfall events can affect the functioning of macrobenthic communities, since flood waters can be rich in sediment or contaminants such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides (Makepeace et al., 1995; Schiedek et al., 2007; Roberts et al., 2008). Heavy rains can also reduce salinity in areas already exposed to high freshwater inputs, such as estuaries, sewage outfalls or storm water drains (Schiedek et al., 2007; Mamboya et al., 2009).

Several studies have shown that early life stages are the most vulnerable to environmental stressors (Piola and Johnston, 2006 and literature within). In particular, the toxicity of copper is known to be stronger in early life stages (e.g. Gledhill et al., 1999; Johnston et al., 2002; Watson et al., 2008), so it should be expected to have marked effects on the initial colonization of marine substrata. Aside from effects on the identity and number of species colonizing substrata, high concentrations of copper could also be expected to change rates of respiration and accumulation of biomass, since it interferes with metabolic activity and growth of organisms (see above). Changes in salinity created by freshwater inputs could also have negative impacts on larval stages and their mobility (Vetemaa and Saat, 1996). Furthermore, it is hypothesized that the presence of fresh water may also increase the bioavailability of copper and therefore its toxicity (Eklund and Kautsky, 2003).

In this context, laboratory experiments are not an ideal system to study interactions among stressors, because, although they allow for controlled delivery of stressors and precise measurements of responses, they lack many of the complex biological and physical processes present in the natural environment (Johnston et al., 2002; Townsend et al., 2008; Roberts et al., 2008; Mayer Pinto et al., 2010). On the contrary, field experiments can be logistically challenging and difficult to interpret, but they give the opportunity to manipulate the stressors of interest while allowing other factors to vary naturally. They are comparatively rare, however, despite the fact that their results are more representative of effects that can be expected in nature (Johnston et al., 2002; Townsend et al., 2008; Mayer Pinto et al., 2010).

In this study, a field experiment was done to test whether copper and freshwater input act independently or interactively to affect the development of marine subtidal assemblages and their functioning (i.e. respiration and total biomass). We tested whether the combined effects of copper and freshwater would be greater, less than or equivalent to their individual effects.

2. Materials and methods

2.1. Study site

The study was undertaken in Malahide marina, just north of Dublin on the East coast of Ireland (53°27.238′N, 006°9.055′W) from April to July 2012. The marina is situated in a narrow shallow estuarine bay at the entrance of the River Broadmeadow.

The marina hosts 350 berths connected to floating pontoons. The walls of the pontoon are made of concrete, and are covered mainly by invertebrate assemblages, dominated by the mussel *Mytilus edulis*, the barnacle *Semibalanus balanoides*, anthozoa of the genus *Metridium* and ascidians. Just below the water's surface, a macroalgal component is also present, composed primarily of *Ectocarpus* spp., *Ulva* spp. and *Ceramium* spp. The inner wall of the southern breakwater was used as the study site.

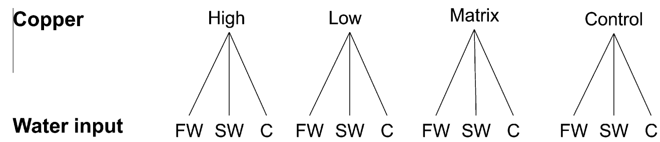

Fig. 1. Graphical representation of the design used to test the combined effect of copper and water inputs on recruitment. The design has two fixed and crossed factors: "Copper" and "Water input". The factor "Copper" has four levels: "High copper concentration"(H) corresponded to an addition of 2.4 g of copper; "Low copper concentration" (L) equal to the addition of 1.2 g of copper, "Matrix" and "Control" (C), in which copper was not added. "Water inputs" has three levels: "Freshwater", in which the recruitment plates were exposed to freshwater inputs; "Seawater", where the recruitment plates were exposed to flows of sea water and "Control", where the recruitment plates were not exposed to any water flow.

2.2. Experimental design

The combined effect of copper and low salinity on tiles' recruitment was tested through a factorial design. The two factors, "Copper" and "Water input", were fixed and orthogonal (Fig. 1). Copper was delivered through antifouling paint applied on a plastic frame (method adapted from Johnston and Webb (2000)). The paint was a mixture of copper powder and a paint matrix. To control for any effect of the paint components on recruitment, the factor "Copper" had 4 levels: "High Copper" (HCu), "Low Copper" (LCu); "Matrix" (M), "Control" (C). The factor "Water input" had three levels: "Freshwater" (FW), corresponding to a continuous exposure to a freshwater flow; "Seawater" (SW), where a constant seawater flow was applied; "Control" (C), in which tiles were simply submerged in water without being exposed to any water flow. The Seawater treatment was included as a control for the possible effects of flow due to delivery of the freshwater as distinct from the reduced salinity it causes.

Four recruitment plates were randomly assigned to each combination of the two factors, for a total of 48 experimental units.

3. Experimental procedure

Each recruitment plate consisted of a $20 \times 20 \times 0.5$ cm tile made of concrete. On each tile the recruitment area (10×10 cm) was delimited by a plastic frame (outer dimensions: 14×14 cm, inner dimensions: 10×10 cm, thickness 0.5 cm), whose purpose was also to deliver copper. The frames selected for the copper treatments were painted with a double coat of an antifouling copper paint: "Copper coat ©". This paint was obtained by dissolving copper powder in a paint matrix: a mixture of a resin (Volatile Organic Component free) and a hardener made of epoxy. In contact with water the painted surface deteriorates progressively and releases copper in the form of cuprous oxide, preventing recruitment and the growth of the organisms. The high copper concentration was established by adding 2.4 g of copper powder to 2.4 ml of matrix per frame (i.e. half of the quantity of copper recommended for general use on boats). The low copper concentration level was obtained by adding 1.2 g of copper per frame. The procedural control involved painting the frame only with the matrix. In the unmanipulated control treatment unpainted frames were applied. The frames were replaced every 3–4 weeks to ensure an effective delivery of copper, as recommended by Johnston and Webb (2000). All the tiles were attached with cable ties to two plastic conduits, which were screwed to the wood sleepers. The tiles were suspended at a depth of 30 cm below the water and separated from each other by at least 1.2 m to avoid any cross contamination (M. Browne et al. unpubl. data).

Four tiles in each levels of copper were randomly assigned to each of the three levels of water input: "Freshwater", "Seawater" and "Control". Freshwater was applied as a constant flow of tap

Please cite this article in press as: Saloni, S., Crowe, T.P. Impacts of multiple stressors during the establishment of fouling assemblages. Mar. Pollut. Bull. (2015), http://dx.doi.org/10.1016/j.marpolbul.2014.12.003