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Impacts of bleach on bryozoans: A framework to distinguish direct and indirect effects using chemical and physical manipulations

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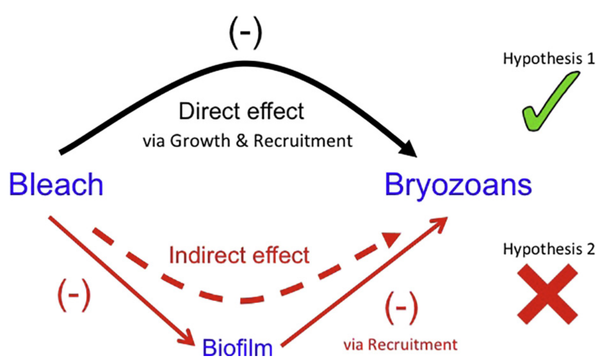
HIGHLIGHTS

- Specific manipulative experiments are necessary to actually test indirect effects of contaminants.
- Physical manipulation of the changing variable as well as the contaminant is necessary.
- I tested the mechanisms by which bleach reduced covers of a marine organism and whether those are direct or indirect.
- Results indicate a negative direct effect of bleach on bryozoans via effects on growth and recruitment.
- This work can be used as a general framework to distinguish between direct and indirect effects of pollutants.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemical disturbances, caused by contamination, are a global issue and can cause changes in the abundance of populations of one or more species via direct and/or indirect effects. This, in turn, can have profound consequences on assemblages and/or systems. Understanding how contaminants affect functional groups or taxa, i.e. which ecological or biological processes are being affected, is necessary to better predict their consequences. To distinguish between direct and indirect effects of contaminants, however, specific experiments, physically manipulating the changing variable (in this case, biofilm) as well as the contaminant, are necessary. Here, I tested which processes were affected by bleach, a biocide commonly found in urban run-offs that caused a decrease in covers of bryozoans. Effects of bleach on recruitment and growth of bryozoans were variable, suggesting that impacts are complex. Nevertheless, results indicate that bleach reduced recruitment of bryozoans. Therefore, manipulative field experiments were done to test whether these effects were direct or indirect, through changes in the abundance of photosynthetic biofilms. Responses of biofilms varied with the duration and exposure to bleach, as well as the timing of experiments. Abundance of biofilms did not seem, however, to affect the number of recruits of bryozoans, which could suggest a direct effect of bleach on bryozoan recruitment. Given that bryozoans are a major component of subtidal benthic assemblages and an important food source for >300 species, decreases in their abundance, as those observed here, might have important knock-on effects on marine systems due to trophic cascades.
Capsule.
In situ manipulative experiments showed that bleach decreased covers of bryozoans, which is possibly due to a combination of effects on their growth and recruitment. Manual removals and chemically induced removals are necessary to disentangle direct and indirect effects of contaminants.

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1. Introduction

Chemical contamination is increasing worldwide (CAS, 2015) and is considered the second greatest threat to a large number of aquatic species, behind only habitat loss (Wilcove and Master, 2005; Rohr et al., 2006; Mayer-Pinto et al., 2016). Contamination from anthropogenic sources is frequently associated with global reductions in biodiversity. This is either as a result of reduced species richness, increased dominance of tolerant species or a combination of both (Johnston and Roberts, 2009). Also, contaminants are linked to impacts on the function of systems (Johnston et al., 2015). As with any detrimental disturbance, contaminants can affect organisms directly, via e.g. effects on survival or growth, or indirectly, via changes in interactions between organisms (e.g. Fleeger et al., 2003; Saborido-Rey et al., 2007). However, we still have limited understanding on which processes are being affected and whether those effects are direct or indirect.

Ecotoxicology studies have increased considerably in the last decades (e.g. Relyea and Hoverman, 2006; Vighi and Villa, 2013). Nevertheless, few studies have been specifically designed to distinguish between direct and indirect effects of chemical disturbances such as contamination (but see e.g. Johnston and Keough, 2002, Johnston et al., 2002, Eeva et al., 2003). Typically, indirect effects are simply inferred from negative correlations between variables without experimentally testing such effects (Mayer-Pinto et al., 2010; Rochman et al., 2016). Experiments to disentangle and identify indirect effects should involve the manipulation of not only the contaminant, but also of at least one of the variables/factors involved in the ecological processes per se (Underwood, 1997). Nutrients, for example, have been linked to impacts on ecosystems through a series of direct and indirect effects in the food-chain (e.g. Hartley et al., 2003; Hjorth et al., 2006; Burkholder et al., 2007). Hjorth et al. (2006), for example, stated that "…bacterial communities showed positive indirect effects with high activities due to higher amounts of available substrate from algal death…". However, to establish that the patterns observed were indeed caused by indirect effects of nutrients through changes in the trophic cascade, it is necessary to (physically) manipulate the amount of available substrate in areas without the presence of the contaminant. If an increase in substrate availability, via experimental manipulation, causes the same effects as the nutrients, than it is possible to affirm that, in fact, the initial pattern observed was due to indirect effects. Without this type of manipulation, however, one can only infer the causes for the high activity in bacterial communities.

Furthermore, the potential impacts of contaminants on organisms and/or assemblages are often investigated in laboratories or in mesocosms, but such studies often are not representative of natural conditions (Underwood and Peterson, 1988; Chapman, 2002; Mayer-Pinto et al., 2010; Vighi and Villa, 2013). Since interactions among organisms and ecological processes can modify responses of organisms to a contaminant (e.g. Johnston and Keough, 2003), laboratory-based toxicological studies typically lack the ability to predict indirect effects of toxicants on organisms mediated through processes that cannot occur in laboratory conditions, such as recruitment (Underwood and Peterson, 1988). Thus, these types of studies often provide limited understanding on the broad ecological consequences of chemical disturbances on ecosystems. Studies done *in situ* are necessary to be able to accurately predict effects of disturbances on ecosystems.

Bacteria, diatoms and micro-algae are the primary colonizers of hard surfaces submersed in the sea and form biofilms, which strongly influence fouling assemblages by providing settlement cues for many species of invertebrates (Henschel and Cook, 1990; Wieczorek and Todd, 1998). Chemical contaminants, such as metals and biocides, can affect biofilms changing their composition and abundance (Sabater et al., 2007; Mayer-Pinto et al., 2011), which can have indirect effects on benthic organisms and/or assemblages. Sodium hypochlorite, commonly known as bleach, is a particular pervasive type of biocide that has been successfully used to control biofouling in industrial seawater cooling systems (Lopez-Galindo et al., 2010; Moreira et al., 2010). Bleach has also been used for >100 years as a disinfectant in households and hospitals and is a common contaminant in runoffs and sewage outfalls close to urban centres (Zoller and Sosis, 2005). This contaminant was therefore used to test hypotheses about direct and indirect effects of chemical disturbances on ecological processes in marine systems.

The main aim of this study was to assess the effects of chemical disturbances via addition of bleach on subtidal fouling assemblages using manipulative field experiments. Although there were no effects on the structure (i.e. composition and relative abundance of species of the entire assemblages, bleach significantly reduced percentage cover of bryozoans. Bryozoans are important components of fouling assemblages worldwide (Gordon and Costello, 2016) and are an important food source for >300 marine species (Lidgard, 2008). Bryozoans also support diverse assemblages of macro-invertebrates and can serve as nursery grounds for some juvenile fish species (e.g. Wood et al., 2012). These results have then led to the subsequent aim of the study, which was to determine whether effects on bryozoans were direct or indirect. The specific hypotheses were that the toxicity of bleach could be directly affecting bryozoans, inhibiting their recruitment and growth, or that the observed reduced cover could be due to an indirect effect, whereby the chemical contaminant could be affecting the biofilm, which in turn, could be affecting the recruitment of bryozoans. To test these hypotheses, experiments where biofilms were physically manipulated on panels exposed to bleach and on control panels (without bleach) were done to decouple the effects of the contaminant on ecological processes from biofilm-mediated effects on such processes, if any. This is the first study to evaluate possible direct and/or indirect effects of bleach on bryozoans.

2. Methods

2.1. Study locations

Experiments were done at two sites ~50 m apart within two locations in Sydney Harbour (Chowder Bay: 33.8397° S, 151.2524° E and Watsons Bay: 33.8398° S, 151.2817° E), totalling 4 sites. These locations were characterised by similar hard substrata benthic assemblages mainly composed of sponges, ascidians, bryozoans, barnacles, oysters and cnidarians.

2.2. Application of disturbance

Bleach was released using plaster blocks. This methodology has been successfully used previously, with studies showing significantly greater concentrations of contaminants near contaminated panels and/or sediment plots than near control ones, indicating a release of contaminant into the water-column and/or sediments over the treatment panels/plots (Morrisey et al., 1996; Lindegarth and Underwood, 1999; Johnston and Keough, 2000; Cartwright et al., 2006; Mayer-Pinto et al., 2011). Control blocks (C; 200 ml) were made of 100 g of dental plaster mixed with 70 ml of water. Fifty ml of biocide (50 $g.l^{-1}$ Sodium Hypochlorite; 30 Seconds Limited, New Zealand) were added to 100 g of plaster and 45 ml of water to make blocks contaminated with bleach (Bl). The concentration used here was similar to that used in other studies that have shown significant ecological impacts to benthic taxa, such as reductions in population abundance and changes in species interactions (Mayer-Pinto and Ignacio, 2015; Mayer-Pinto et al., 2015). Settlement panels (11 cm × 11 cm × 0.5 cm of roughened black perspex) were glued to lids of 250 ml plastic sample jars (internal diameter 67 mm). The jars, which contained the plaster blocks, were fixed vertically to weighted 2.1 m long timber beams attached to wharfs. Panels were submerged about 0.5 m deep, facing downwards. Eight 6 mm holes were drilled through the lids and panels to allow a slow release of the contaminant, preventing it from being washed away too quickly (Fig. 1). Every two weeks, the holes were cleaned and plaster blocks