



Enhancing passive sampling tools for detecting marine bioinvasions

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ABSTRACT

Early detection is important for successful management of invasive species, but optimising monitoring systems to detect multiple species from different taxonomic groups remains a major challenge. Settlement plates are often used to monitor non-indigenous marine species (NIMS) associated with vessel biofouling, but there have been few assessments of their fitness-for-purpose. We deployed arrays of settlement plates (“settlement arrays”) containing combinations of treatments that reflected conditions associated with the vessel transport pathway (i.e., copper based antifouling coatings, shaded habitat) to determine the treatment combinations that maximised NIMS diversity. Horizontal (shaded) treatments preferentially sampled higher NIS diversity than vertical plates. Although plates with copper-based biocides had larger proportions of NIS to indigenous species, they sampled only a subset of NIS diversity. Overall diversity was greatly enhanced through use of multiple treatments, demonstrating benefits of multi-faceted sampling arrays for maximising the potential taxonomic and species richness.

1. Introduction

Increasing shipping volumes and diversification of global trade networks have opened many biogeographical boundaries impassable by natural dispersal and promoted the spread of non-indigenous species (NIS) (Ruiz et al., 1997; Hulme, 2009). Consequently, exponential increases in the non-indigenous component of biodiversity is well illustrated by invasion records from many ecosystems, despite potential confounding factors in the process of species discovery (Solow and Costello, 2004; Wonham and Pachevsky, 2006). With the tightening of regulations associated with ballast water discharges (Davidson and Simkanin, 2012), vessel biofouling remains a dominant vector of NIS transport (Hewitt and Campbell, 2010). Niche areas of vessels that have ineffective antifouling coatings or which are exposed to less turbulence than flat hull surfaces contribute disproportionately to the level of vessel biofouling (Inglis et al., 2010; Davidson et al., 2016; Moser et al., 2017). Concomitantly, biofouling NIS are often tolerant of shade (Dafforn et al., 2015) and of the biocides in antifouling coatings (Floerl et al., 2004; Piola and Johnston, 2008; McKenzie et al., 2012). Targeting biological traits associated with characteristics relating to vessel-based habitats may provide clues for optimising sampling for NIS transported by these vectors.

Passive sampling of marine organisms using settlement plates has a long history in marine biological research where they have been used to study the recruitment of sessile marine organisms from planktonic life stages (e.g., larvae, spores) into benthic juvenile or adult phases (Coe,

1932; Scheer, 1945; Allen and Wood, 1950; Ryland, 1974; Butler, 1986). In more recent times settlement surfaces have been used to study the demography, life history and ecological processes that contribute to the success of non-indigenous marine species (Tyrrell and Byers, 2007; Floerl et al., 2012a), and to monitor non-indigenous species (Marshall and Cribb, 2004; Labowitch and Cribb, 2006; Gartner et al., 2016). Artificial surfaces sample very broad taxonomic groups, and can preferentially sample specific groups when deployed in different configurations (e.g., vertical vs. horizontal deployment; Knott et al., 2004). However, the ability to preferentially sample different taxonomic groups through various treatments presents an opportunity to combine multiple characteristics into arrays of plates (hereafter referred to as “settlement arrays”) for maximising total biodiversity and NIS biodiversity.

While settlement surfaces target the early life-history stages of marine biofouling organisms, the detection of new NIS incursions is dependent upon the size of the founding population, the fecundity of that population and the intensity and distribution of sampling (Hayes et al., 2005; Inglis et al., 2006a; Inglis et al., 2006b; Harvey et al., 2009). Newly arrived NIS are initially rare in their new environment, posing significant challenges to early detection (Hayes et al., 2005; Inglis et al., 2006a; Inglis et al., 2006b). Because of this, most NIS discoveries occur once they have established and attained ecologically significant densities (Myers et al., 2000; Bax et al., 2002). The probability of detecting any single species within a survey will depend on its level of rarity and dispersion within the sampled environment, the

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sensitivity of the sample method, and the intensity of sampling. Thus, single species surveys can be optimised by specifying a level of rarity (design prevalence) that the survey is intended to detect, by using sample methods that have known (high) sensitivity for the target organism and by adjusting sample effort to achieve a desired confidence (“power”) that the organism will be sampled if it is present in the study area (McArdle, 1990; Cannon, 2001; Hayes et al., 2005). In contrast, where the objective is to sample multiple species or taxonomic groups using a single sample method, optimisation must account for the variable sensitivity of the method for the range of organisms that are targeted (Morrisey et al., 2012; Whittle et al., 2013). Optimising the use of passive sampling devices for biofouling species requires some understanding of how well they sample key taxonomic groups typically associated with vessels (e.g., ascidians, bryozoans, crustaceans, molluscs and algae) and how overall species richness within these taxa is sampled by various plate configurations.

Specific habitat requirements greatly influence the selection of microhabitats by the motile larvae of many sessile invertebrate species (Ryland, 1974; Keough and Downes, 1982), or the survival rates of species with little or no ability to select the substrata which they encounter (e.g., macroalgae). Marine NIS have been shown to settle in high densities on artificial substrata (e.g., wharf piles and pontoons) compared to natural substrata (e.g., rocky reef; Glasby, 1999; Glasby et al., 2007; Tyrrell and Byers, 2007; Dafforn et al., 2012). Explanations for this well-established pattern include the proximity of NIS vectors to artificial structures (Glasby et al., 2007), the placement of infrastructure within degraded environments (Dafforn et al., 2009), covariables associated with artificial substratum, such as the frequent provision of shaded surfaces in modified environments (Bax et al., 2002; Dafforn et al., 2015) and the absence of natural predators (Atalah et al., 2014). Furthermore, tolerance to copper associated with modern antifouling coatings can act as a selective force on the settlement (Floerl et al., 2005), translocation (Piola and Johnston, 2006), and success of NIS in harbour environments typically exposed to high copper concentrations (Piola and Johnston, 2008). Therefore, using settlement arrays designed to target these characteristics (i.e., shade tolerance, copper tolerance, or potential affinity for artificial substrata) may enhance their utility for sampling NIS.

Our aim was to experimentally manipulate several characteristics of settlement plates to sample the greatest overall diversity of biofouling NIS. This included using treatments that favoured particular traits (e.g., shade tolerance, Bax et al., 2002; Dafforn et al., 2015; and biocide tolerance, Floerl et al., 2005; Piola and Johnston, 2006, 2008) that are common among vessel biofouling NIS and treatments that increased within and between treatment biodiversity (e.g., surface heterogeneity that also provided refuge from predation, Walters and Wethey, 1991, 1996). We examined the combination of treatment conditions that would sample; a) the greatest species diversity, and b) the greatest proportion of NIS.

2. Methods

2.1. Experimental design and settlement array deployment

Settlement arrays were deployed in one of New Zealand's largest marinas for recreational vessels, Westhaven Marina, Waitemata Harbour, Auckland, New Zealand. A pilot deployment from 22 October 2014 till 28 January 2015 was used to develop methodologies for two subsequent deployments. From this pilot study, two traits, paint thickness and habitat complexity were modified to further reduce paint thickness and increase habitat complexity (relative to the pilot study). The two subsequent experimental deployments in austral winter (23

June 2015 to 20 October 2015), and summer (12 November 2015 to 16 February 2016) used identical methodologies. The pilot study was not included in the analysis.

The percentage cover of biofouling and richness of NIS and indigenous species were compared among three orthogonal experimental treatments. The treatments were: (1) the presence/absence of thin-layer antifouling coatings (three levels), (2) panel orientation (vertical and horizontal), and (3) surface texture (smooth and pitted). Thin-layer antifouling coatings were used to simulate coatings with degraded biocides that would potentially favour the recruitment of copper tolerant NIS (McKenzie et al., 2012). The three levels within this treatment consisted of a non-toxic control (coated only with primer paint, International Primocon™), plates that were painted with the primer and a single thin layer of 50% diluted antifouling paint (40 µm thick), International Micron Extra™ (“A0.5”), and plates coated with the primer and one thin layer of full-strength antifouling coating (75 µm thick), International Micron Extra™ (“A1”). The thin-layer biocidal treatments represented one-quarter (A0.5) and one-half (A1) of the coating thickness recommended by the manufacturer and were applied to plates using rollers.

The pitted treatment was incorporated based on evidence that small surface irregularities (< 500 µm) can inhibit the recruitment of a range of biofouling species (Bers and Wahl, 2004), but larger irregularities (> 2 mm) can enhance recruitment by providing refuges to predation at vulnerable life-history stages (Walters and Wethey, 1991, 1996). Pits were routed into the half of the plates (22 × 4.5 mm diameter by 2 mm deep), with the other half left smooth. The arrangement of pits was intended to spread the treatment evenly across the settlement plates. Antifouling coatings were applied to the inside of the pits.

The settlement plates were made of 4.5 mm thick PVC (Poly Vinyl Chloride) cut into 145 × 145 mm squares. Plates had two holes 4 cm apart in the centre of the plate for attachment to the frame (note these are separate from the “pitted” treatment). A single plate of each treatment condition (12 plates in total) was secured with zip-ties to each of 10 replicate array frames. The frames were constructed of 32 mm thick PVC pipe, joined with elbow connectors and T-connectors to form three rows, with four plates secured per row (i.e., $n = 10$ plates per treatment, total of 120 plates per deployment). Plates were fitted in alternating horizontal, facing downwards and vertical orientation, with other experimental treatments randomly assigned throughout the frame. To maintain the arrays in a horizontal position, a 1 m length of stainless steel rod was fixed through the centre of each PVC frame, with a 2 m length of chain attached to the cinder-block anchor to hold the frame level throughout the tidal cycle. Arrays were deployed at a constant depth of 2 m, attached to floating pontoons with rope, and secured to the seafloor with chain and a cinder block.

2.2. Biofouling identification

All plates were retrieved after ~3 months deployment and transported to the laboratory in individual bags filled with seawater. Percentage cover of biofouling and species richness were determined under light microscopy. The percentage cover of each individual biofouling species was estimated by coverage of each species at systematic points (10 × 10 cm grid) at 10 × magnification. Abundant species were removed during processing to determine secondary cover of biofouling organisms. Biofouling attached to the sides (including the insides of attachment holes used for attaching plates) or backs of plates were excluded from identification and analysis, thereby considering only biofouling that could attach directly to antifouling coatings. We also measured the time it took to process each plate to provide a measure of the labour costs associated with each experimental treatment.

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