Settlement plates as monitoring devices for non-indigenous species in marine fouling communities

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Abstract
Non-indigenous species (NIS) are one of the leading forces of change in coastal marine ecosystems and are often associated with fouling communities, especially the artificial structures of marinas and ports. As a result, monitoring of marine fouling communities is crucial to evaluate the introduction and spread of NIS as well as assess the efficacy of legislation aimed to prevent further introductions. Settlement plates have long been used as a means of studying fouling communities. Many factors such as orientation, movement, and substrate type have been shown to influence the number and type of organisms present in fouling communities, but one key question remains poorly studied: how well do settlement plates represent the established fouling community of a marina, especially regarding NIS? We investigated this question, by examining the sessile invertebrates on both marina structures and settlement plates from three marinas in San Francisco Bay (California, USA). Total species richness, NIS richness, and community composition on settlement plates were found to be similar to those on existing marina floating docks. Our results indicate that settlement plates can provide a sensitive and standardized measure of the NIS richness and composition in fouling communities.

Key words: community composition, fouling communities, non-indigenous species, settlement plates, San Francisco Bay

Introduction
Non-indigenous species (NIS) are a leading threat to biodiversity and have a diverse range of effects (Ruiz et al. 1997; Stachowicz et al. 1999; Carlton 2001; Bax et al. 2003). Once established, NIS may be capable of out-competing native species for food, habitat, and other resources, thereby potentially changing the established community structure (e.g. Fritts and Rodda 1998, O’Dowd et al. 2003), altering ecosystem function (Vitousek et al. 1987), and contributing to the decline of threatened and endangered species (Gurevitch and Padilla 2004). Competition by NIS could lead to the elimination of native species and creating greater homogenization of biodiversity (Levine 2000; Byers 2002). Human activities, such as urbanization, commercial and recreational shipping, and the aquaculture, pet, and ornamental trades, have resulted in the introductions of NIS throughout the world leading to major changes in species distribution (e.g. Williams et al. 2013).

A major influence humans have on coastal ecosystems is the construction of artificial habitats, such as in marinas and ports. These structures provide suitable hard-substrate habitat, in areas that may not otherwise have them, for a variety of epifaunal fouling organisms (Connell 2001; Ruiz et al. 2009). These artificial structures act as a point of entry for NIS as they are released from ballast water and ship hulls (Bax et al. 2003; Fofonoff et al. 2003a). Consequently, these structures support an increased richness and abundance of NIS compared to naturally occurring hard substrates such as nearby reefs of rocks, shellfish, or polychaete tubes (Wasson et al. 2005; Glasby et al. 2007; Tyrrell and Byers 2007; Dafforn et al. 2012). Thus, as hotspots, marinas and ports are ideal focal points for early detection of NIS, which is critical for potential eradication and effective management.
Marinas and ports are made up of a variety of materials not found in natural shorelines, such as plastic or foam, which not only provide new increased habitat but may also favor NIS (e.g. Tyrrell and Byers 2007). Many previous studies have investigated how the abundance and composition of fouling organisms on artificial substrates vary in response to numerous factors including: orientation (e.g. Glasby 2001; Glasby and Connell 2001), movement (Glasby 2001), light exposure (Glasby 1999), distance from the seafloor (Glasby 1999), surface composition and texture (e.g. Pomerat and Weiss 1946; Raimondi 1988; Anderson and Underwood 1994; Tyrrell and Byers 2007), size (e.g. Jackson 1977; Keough 1984), and color of the substrate (e.g. Pomerat and Reiner 1942; James and Underwood 1994), as well as numerous interactions of these components. A common method for investigating fouling communities is to deploy settlement plates to act as a standardized, passive settlement sampling devices (e.g. Sutherland and Karlson 1977; Glasby 1999; Stachowicz et al. 1999), which have been widely adopted in studies of fouling communities and surveys for NIS worldwide (e.g. Sutherland 1974; Bax et al. 2003; Blum et al. 2007; Tracy and Reys 2014; Marraffini and Geller 2015).

Given the important role of artificial substrates in the introduction and establishment of NIS, monitoring these areas is likely to be an efficient use of resources to detecting novel introductions as well as monitoring the ranges of introduced species. Our goal was to compare two common methods, settlement plates and diver surveys, for sampling marine epifaunal communities and NIS. We hypothesized that settlement plates and dock scrapes would perform similarly in assessing the total species richness, NIS richness, and community composition of the resident floating dock community given their similarities in materials and orientation.

Methods

As part of a larger fouling community study, we sampled three marinas in San Francisco Bay, California, USA: Richmond Bay Marina, San Francisco Marina, and San Leandro Marina (Figure 1). Settlement plates (hereafter referred to as plates) consisted of bare, dark gray, lightly sanded PVC plates measuring 13.7 × 13.7 cm, attached to bricks with the experimental surface facing downward parallel to the seafloor, to mimic floating docks. Plates were suspended one meter below randomly chosen floating docks in each marina (Blum et al. 2007; Crooks et al. 2011) for three months, during the summer (June to September), to coincide with the period of high seasonal recruitment and provide sufficient time to develop mature, communities (Ruiz et al. unpublished data). Diving surveys scraped the material inside a 15.5 × 15.5 cm quadrat haphazardly placed against the horizontal underside surface of randomly selected floating docks. Although the sampling area differed, the slightly larger area of the dive quadrat allowed for a greater chance of containing more species, thereby providing a conservative measure of relative performance of plates. The depth of floating dock samples was dictated by the dock’s construction, but was approximately 40 cm below the surface in all locations. All plate retrievals and diver sampling took place within a few weeks of each other in September of each year (Sept. 14–30, 2009 and Sept. 7–17, 2010), with 5–25 samples of docks and plates taken in each marina each year (Table 1).

All sessile invertebrate organisms from both survey methods were collected and brought to the lab for identification (dive samples as scrapes and plates were collected whole). In both sampling techniques all organisms collected were examined live under a dissecting microscope. We identified each morpho-species to the lowest possible level based on morpho-
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Table 2. Analysis of Variance (three way ANOVA) for each response variable. Datasets were examined individually.

| Docks and Plate Comparison | Response | Predictor | Deg. Of Freedom | Sum of Squares | Mean Squares | F value | P value |
|----------------------------|----------|-----------|-----------------|----------------|--------------|---------|---------|
| Total                      | Survey   | 1         | 4.817           | 4.817          | 0.389        | 0.534   |
| Total                      | Year     | 1         | 137.811         | 137.811        | 11.124       | 0.001   |
| Total                      | Site     | 2         | 80.173          | 40.086         | 3.236        | 0.044   |
| Total                      | Survey:Year | 1     | 0.797           | 0.797          | 0.064        | 0.800   |
| Total                      | Survey:Site | 2    | 0.186           | 0.093          | 0.008        | 0.993   |
| Total                      | Site:Year | 2     | 4.989           | 2.494          | 0.201        | 0.818   |
| Total                      | Survey:Site:Year | 2    | 74.8631         | 37.431         | 3.021        | 0.053   |
| Total                      | Residuals | 96      | 1189.281        | 12.388         |              |         |
| NIS Richness               | Survey   | 1         | 1.45            | 1.452          | 0.259        | 0.612   |
| NIS Richness               | Year     | 1         | 12.08           | 12.082         | 2.159        | 0.145   |
| NIS Richness               | Site     | 2         | 68.61           | 34.304         | 6.129        | 0.003   |
| NIS Richness               | Survey:Year | 1    | 16.63           | 16.628         | 2.971        | 0.088   |
| NIS Richness               | Survey:Site | 2    | 12.00           | 6.000          | 1.072        | 0.364   |
| NIS Richness               | Site:Year | 2     | 1.98            | 0.988          | 0.176        | 0.838   |
| NIS Richness               | Survey:Site:Year | 2    | 27.81           | 13.907         | 2.485        | 0.088   |
| NIS Richness               | Residuals | 96      | 537.29          | 5.597          |              |         |

loggy (for convenience, we hereafter refer to morphospecies as species). Total richness is used to refer to all species, regardless of invasion status (native, non-indigenous, cryptogenic, and unresolved), while NIS richness refers only to species classified as non-indigenous; these designations are based on available literature and the National Exotic Marine and Estuarine Species Information System database (NEMESIS; Fofonoff et al. 2003b). Cryptogenic species refer to those that are not demonstrably native or introduced (Carlton 1996), while unresolved species are those that could not be identified to a species level and therefore could not be assigned a native status (except Botryllinae as there are no known native species within this family on the west coast of North America).

We performed a three-way ANOVA on untransformed data to compare total species and NIS richness between plate and floating dock samples. All assumptions of the ANOVA were met (Leven’s Test, $α = 0.05$; visual assessment of residuals for normality and linearity; and independence of samples). This model compared survey type (dock or plate), site (marina/location), year, and the interaction of these main effects. We then compared community composition on the plates and docks with a Permutational Multivariate Analysis of Variance (Adonis function in Vegan Package; Bray and Curtis 1957, Clarke 1993) comparing presence and absence of species to determine how community composition differed between surveys and sites (using Year as a nesting factor). Statistical analyses were performed in the R environment for statistical computing (R Core Team 2015) using the vegan package (Oksanen et al. 2017) and stats package (R Core Team 2015). Samples that contained fewer than two species were removed from community analyses, this only removed one sample from the examination of NIS community composition.

**Results**

Across all sites and sampling events, we collected a total of 108 taxa of sessile marine invertebrates from seven phyla. Of these 89 (82.4%) were identified to genus or species level including 27 (24.7% of total taxa) classified as NIS (Supplementary material Table S1). Cryptogenic taxa were assigned to genera or families (Carlton 1996). Some of these represent higher order taxa and may actually overlap if the species could be identified with a higher degree of confidence (5 and 6 taxa respectively; Table S1). Twenty-four species were found to be unique to plates while 26 separate species were unique to docks. Some of these represent higher order taxa and may actually overlap if the species could be positively identified with a higher degree of confidence (5 and 6 taxa respectively; Table S1). Bryozoa and Urochordata represent the most species rich phyla observed with 29 and 36 taxa respectively. The dock and plate samples showed a similar level of uncertainty with respect to the identity of taxa within Bryozoa, while overall plates had a slightly better resolution of taxa with 12 unique unresolved taxa compared to 15 unique unresolved taxa on docks (unique meaning not found in the other survey method, several other unresolved taxa were found on both survey methods; Table S1).

**Total richness**

Total species richness did not differ between floating docks and plates ($F = 3.89$, $p = 0.534$) but did vary between sites (Table 2; Site $F = 2.628$, $p = 0.043$, Figure 2), post-hoc tests revealed that San Leandro Marina
Average richness found in each sample for both richness measurements. Error bars are equal to ± 1SD. Total species includes taxa from all native statuses.

was significantly different from San Francisco Marina (p = 0.033) while neither differed from Richmond Marina. Year significantly influenced the total species richness (F = 11.21, p = 0.001, Table 2), and we found no interaction with survey type.

**NIS richness**
Among the species found in surveys across all sites, 27 were NIS (24.7%), of which 23 (27.4%) were found on plates and 22 (26.2%) on floating docks. We found no differences among average NIS richness between floating docks and plates or between years studied (Table 2, Figure 2, p > 0.05). Site (marina location) was the only main factor found to significantly influence NIS richness (using both methods of counting taxa p < 0.005, Table 2).

**Community composition**
Overall community composition varied between plates and floating docks (Figure 3; PERMANOVA F = 3.64, p = 0.001); and communities showed greater differences among sites (PERMANOVA F = 12.57, p = 0.001). The composition of NIS showed a similar pattern, survey type significantly altered community composition (PERMANOVA F = 3.16, p = 0.004, Figure 4) as well as site (PERMANOVA F = 18.46, p = 0.001). Both analyses showed that site (or location within San Francisco Bay) had a much larger effect on community composition than survey type. Upon further examining the communities, two NIS, *Schizoporella japonica* and *Corella inflata*, were found only at San Francisco Marina, on both plates and existing marina structures (Table S1).
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Discussion

We found that species richness (both overall and NIS) was comparable between plates and dock scrapes for the fouling communities of San Francisco Bay. While NIS and total community composition varied across survey types this effect was small compared to that of sampling locations suggesting that both sampling methods were similarly effective at monitoring the community since there was a large overlap in the species composition among the sampling methods. Understanding the effectiveness of commonly used survey methods to provide information about NIS, particularly high risk areas such as marinas, is fundamental to the management of marine biological invasion. Our results align with previous literature (Glasby 2001; Glasby and Connell 2001; Glasby et al. 2007) and our predictions to show that plates perform well as a survey method. While this result is expected for total species richness, our work here confirms its validity in a new area and supports the extension of this pattern to the NIS community within San Francisco Bay.

In this study, we found that plates collected additional species and NIS not found in dock samples (5 NIS compared to 3 NIS unique to docks), further supporting their use as monitoring devices for newly arriving NIS or those NIS with patchy distributions on existing marina structures which may be missed during diver surveys. While additional sampling of docks may have captured these species, monitoring through plates offers an alternative to intensive diver surveys. Despite the fact that age of the communities sampled were different we did not observe major differences in the richness of the communities and only small differences in the composition.

Figure 3. Non-metric Multidimensional Scaling representation of the total community composition Dock and Plate samples based on Bray similarity matrix. This includes all taxa (putative taxa measurement) and all native statuses.
Figure 4. Non-metric Multidimensional Scaling representation of the NIS community composition Dock and Plate samples based on Bray similarity matrix. This includes all taxa (putative taxa measurement) and only those classified as NIS.

(Figures 2–4). Though year and sites were significant factors in this study, we believe that result is likely due to background recruitment patterns that exhibit inter-annual and spatial variation (Chang et al. unpublished data). While our study may show specific patterns in species and richness distribution our intent was to highlight the validity of settlement plate for their use in monitoring the general fouling and NIS community.

Settlement plates offer multiple benefits as a method for monitoring fouling communities. They provide a standardized method for monitoring artificial habitats allowing for comparisons across time and space (e.g. Freestone et al. 2013; Ruiz and Hewitt 2002; Ruiz and Carlton 2003; Leray and Knowlton 2015); provide a sensitive measure of invertebrate species on artificial substrates; and can be less labor intensive than dive surveys of existing marina structures, as well as provide an easily accessible substrate for experimentation. Plates also provide a more easily observed collection of species, while some organisms, especially those that are particularly small or rare, may be lost in scrapings. While plates are likely to fail to collect species that are restricted to a particular habitat, especially habitats other than artificial shallow-water substrata, our results demonstrate that plates are effective for the monitoring of NIS and provide a valuable research method to evaluate community composition in floating artificial habitats.

Monitoring of marine fouling communities is crucial to evaluate the introduction and spread of NIS as well as assess the efficacy of legislation aimed to prevent further introductions. For research and management purposes, plates allow scientists to efficiently and effectively evaluate invasion dynamics,
including both the detection of newly introduced NIS through continued monitoring and possible local-to-regional interactions with other forcing functions. With policies advancing at international, national, and regional levels to limit new introductions, including those associated with the ballast water and hull fouling of commercial vessels, government agencies and scientists have been tasked with evaluating the performance of current management actions (69 Fed. Reg. 44952; Frazier et al. 2013), leading to the need for effective and validated sampling methods. The results of our study support the use of settlement plates as monitoring devices for NIS in the marine fouling community as part of both management and scientific inquiries.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Taxa recorded during each sampling event in San Francisco Bay in 2009–2010. This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2017/Supplements/MBI_2017_Marraffini_etal_Table_S1.xlsx