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Research article

Do we need to jump in? A comparison of two survey methods of exotic ascidians on docks

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Abstract

Many ports and marinas contain exotic ascidians, ostensibly because they offer adequate physical habitat and harbour many boats or ships, which are considered important vectors of exotic ascidians. Standardized quantitative sampling of these habitats would be useful to assess exotic ascidian presence and abundance, as well as for more detailed ecological studies. In this paper two methods for sampling exotic ascidians are compared using data from nine marinas along the Olympic Peninsula and Upper Puget Sound, Washington, U.S.A. One method is considerably less expensive, drier and easier: it consists of laying a simple grid over the side of the floating dock from the surface, with species identity and abundance being measured on the spot. The other method involves swimming underneath marina floating docks, taking standardized digital photographs and analyzing the photographs later. Differences between these two methods might be expected because the environment sampled, the underneath versus the sides of floating docks, is slightly different, especially with respect to light. However, this study finds that both methods observed almost identical presence/absence patterns for exotic ascidians and, at least for the two most common exotics *Botrylloides violaceus* and *Diplosoma listerianum*, observed similar abundance trends across sites. Further analyses show that, while there are differences in the overall communities observed by the two methods, community patterns are correlated. Overall, this study finds that the easier surveys of the sides of floating docks are as effective for rapid assessment of exotic species presence/absence and relative abundance as those of the undersides of floating docks.

Key words: ascidian, *Botrylloides violaceus*, *Diplosoma listerianum*, *Styela clava*, exotic species survey, surveying floating docks, fouling community

Introduction

Docks and pilings found in ports and marinas often harbour populations of one or more exotic ascidian species. Possible reasons for this include the availability of suitable hard substrate provided by these establishments, designs that often result in the retention of locally-produced propagules (Floerl and Inglis 2003) and the traffic of boats with hull fouling, which is considered a major vector of exotic ascidians (Wonham and Carlton 2005; Floerl and Inglis 2005 and Lambert 2007). Given the ubiquity of exotic species in ports and marinas, and the fact that ports and marinas are becoming increasingly numerous as human populations expand along coastal areas (Gray 1997), it is unsurprising that floating docks have been sites for many experimental studies investigating exotic ascidians (e.g., Agius 2007; Blum et al. 2007). Yet, despite the abundance of exotic ascidians in ports and marinas, there have been few published systematic surveys of these habitats (but see Lambert and Lambert 2003). This is unfortunate because standardized surveys of dock fouling across large geographic regions could be an inexpensive and simple means to gather data that would be useful to resource managers and scientists alike. Regular surveys of dock fouling communities could help to rapidly detect new introductions, track fluctuations in abundance over time and investigate important invasion pathways. They would also be valuable to many types of ecological studies aimed at disentangling factors influencing exotic ascidian establishment and success across space or time. Standardizing such surveys would facilitate data exchange between investigators, thus enabling comparisons and conclusions to be drawn across a broad geographic scale.

This study aimed to assess accurate and easy exotic ascidian sampling protocols for docks. Two survey methods were compared to see if their results of exotic ascidian presence/absence and abundance estimates were similar. One method can be conducted from land, requires little equipment and can be completed in a couple of hours. This method only samples the sides of docks. Previous studies have shown that well-lit areas such as these often support different fouling communities than shaded areas (Glasby 1999). Another method sampled the undersides of docks using an underwater camera. Sampling underneath floating docks required more money and equipment for taking underwater photographs, as well as more time for analyzing the photographs afterwards. Furthermore, species hidden underneath other organisms would be undetectable in photographs. It should be noted that neither of these methods was meant to provide an exhaustive search for exotic ascidians in marinas, so they probably do not sample all exotic ascidian species likely to be present at a site. The concrete floating docks were sampled in a random, repeatable fashion in order to compare sites, rather than to thoroughly search a site for exotic species. The two methods used here would probably miss exotic species present in very low abundance, those that prefer non-concrete substrates or those that prefer depths greater than one meter.

The goal of this study was to determine if the exotic ascidian populations and native fouling communities observed by these two methods are similar. This study also indirectly tested whether the fouling communities on the sides and undersides of docks are similar. Patterns of exotic ascidian presence and abundance observed with each method were compared using parametric and non-parametric regressions. Fouling community patterns were compared using cluster analysis and Mantel tests. After determining the equivalence of the two methods, their advantages and disadvantages are discussed more critically.

Methods

From-the-Docks Survey (FTD)

Nine marinas with floating concrete docks were chosen within the Strait of Juan de Fuca/ Upper Puget Sound region of Washington, USA and surveyed between September 14^{th}-19^{th}, 2005 (Figure 1, Annex 1). At each marina a 20cm× 20cm quadrat with a 4cm×4cm grid was suspended from the surface of the water parallel to the side of the floating dock and ~10cm below the waterline. The identity, number of individuals and percent cover of species were estimated in 8 random quadrats per site. Observations were made without interfering with or moving any of the organisms.

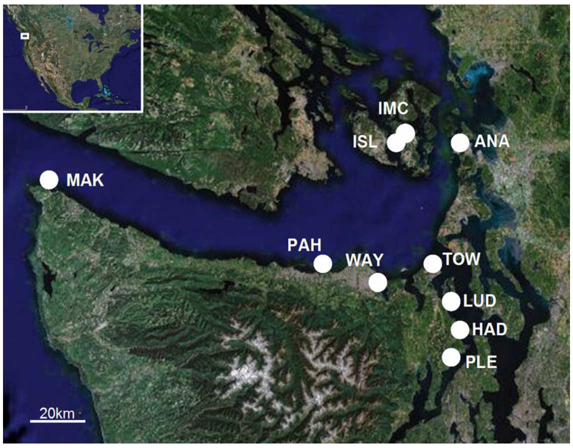

Figure 1. Map of survey sites along the Strait of Juan de Fuca and Upper Puget Sound of Washington, USA. Each site is labeled with a 3-letter code. See information on survey sites in Annex 1. This map created using Google mapping service 2007 (http://www.maps.google.com).

Under-the-Docks Survey (UTD)

After completion of each FTD survey, twelve photographs were taken of a 20cm*20cm area at random locations underneath the docks of the marina. Photographs were taken with an Olympus C-5060 Wide Zoom digital camera, using the 'macro' setting and two external INON D-180S underwater strobes. To ensure that all photographs taken were of equal area and resolution, the camera and strobes were mounted on a stand made of ¼ inch PVC rods. Stand specifications can be obtained by contacting the author. To take the pictures, a swimmer would travel at least 3m from the previous photograph location, hold the stand securely against the underside of the dock and take a photograph. Photographs were checked for clarity on the LCD screen immediately afterwards; if the photograph was obstructed or out-of-focus, the sample was repeated at a nearby (<25cm away) location. For analysis, 12 photographs from each site were randomly chosen, displayed on a 48cm LCD computer monitor and overlain with a grid of 1% cover blocks in GIMP v2.4 (http://www.gimp.org/release-notes/gimp-2.4.html). The identity and number of individuals were recorded, and the percentage cover was visually estimated for each species. All photographs were analyzed by the author in random order. In both methods, taxa were classified to the lowest possible taxonomic unit, which was generally to species level but in some cases (e.g. sponges) coarser. Both surveys were conducted and analyzed by the author.

Comparison of methods

For each method at each site, species presence/absence was recorded and the mean percentage cover was calculated, rounded up to the nearest integer. Presence/absence and abundance (percentage cover) tables were created for each site and each method. A null hypothesis of no relationship between the percentage cover values estimated by FTD and UTD methods at each site was tested using one non-parametric (Kendall's τ) and one parametric test (linear regression, Pearson's r) using the R software for statistical computing (http://www.r-project.org). Only *Botrylloides violaceus* Oka, 1927 and *Diplosoma listerianum* Milne-Edwards, 1841, were found at a sufficient number of sites to allow for the use of parametric statistical tests, but results for other species are shown for heuristic value.

To compare the community patterns, Bray-Curtis dissimilarities were first clustered using the unweighted paired-group method (UPGMA) to visually inspect similarity patterns. Then a null hypothesis of no correlation between the dissimilarity matrices of the FTD and UTD methods was tested with a Mantel test with 5000 permutations. Since there is some debate over the best distance metric to use for species abundance data, the Mantel test was performed using 3 other common metrics: Jaccard, Morisita and Euclidean distances. These analyses were performed in the Past v1.77 statistical package (http://folk.uio.no/ohammer/past/).

Results

A total of 67 species were recorded, which included 17 ascidian species. Of these ascidians, 3 species are known to be exotic to the region: *Botrylloides violaceus*, *Botryllus schlosseri* Pallas, 1766 and *Styela clava* Herdman, 1882. One other species, *Diplosoma listerianum*, is conservatively considered cryptogenic in this region (G. Lambert, pers. comm.) although it is likely exotic and will here be grouped as such. *B.violaceus* was the most widespread and abundant invasive ascidian, occurring in all marinas surveyed to date and at some sites covering over 10% on average of available space (Table 1). *D. listerianum* occurred in all but one marina and was usually abundant. *B. schlosseri* occurred at two sites in low abundance, while *S. clava* occurred at only one site (Pleasant Harbor) but in relatively high abundance.

Both survey methods detected the same set of exotic species among sites (Table 1), except for two sites where *D. listerianum* was present in the FTD survey but not in the UTD survey. For percentage cover, only two species (*B. violaceus* and *D. listerianum*) were present at enough sites to allow statistical testing. For these species, and the sum of all exotic ascidian species, the null hypothesis of no correlation between survey methods could be rejected using both parametric and non-parametric tests (Table 2). Interestingly, the FTD surveys consistently showed higher percentage cover of exotic ascidian species (Figure 2), except in the case of *S. clava* (Table 1).

Community pattern analyses suggest that the two survey methods observe slightly different communities, but that the communities observed at each site with each method are highly correlated. The dendrogram produced by cluster analysis (Figure 3) had a cophenetic correlation of 0.79, implying a good fit with the original pair-wise distance between sites and methods. If the same presence/absence and relative abundances of species were observed by the two methods, then sites should cluster together. This only occurred at 2 sites (PLE and ANA), but other sites tended to cluster near each other. Mantel tests using a different distance metrics all showed significant (Bray-Curtis $p=0.002$, Jaccard $p=0.018$ and Euclidean $p=0.043$) or near-significant (Morisita $p=0.067$) correlations of the dissimilarity matrices at the $p=0.05$ level, indicating that the two methods observed similar community patterns.

Discussion

This study shows that the easier and quicker FTD assessment of exotic ascidian cover is comparable to the more labour-intensive underwater surveying (UTD) method. Both UTD and FTD methods observed very similar presence/absence patterns and trends in percent

Table 1. Average percent cover of invasive ascidian species among sites using UTD and FTD surveys. Bv = *Botrylloides violaceus*; Bs= *Botryllus schlosseri*; Dl = *Diplosoma listerianum*; Sc= *Styela clava*; Total = Bv + Bs +Dl +Sc

Site	UTD					FTD				
	Bv	Bs	Dl	Sc	Total	Bv	Bs	Dl	Sc	Total
ANA	2	0	0	0	2	14	0	0	0	14
HAD	1	0	2	0	3	3	0	1	0	4
IMC	1	0	2	0	3	6	0	7	0	13
ISL	1	0	2	0	3	9	0	7	0	16
MAK	1	0	1	0	2	2	0	1	0	3
LUD	1	0	2	0	3	4	0	0	0	4
PLE	7	4	1	8	20	17	6	0	6	29
TOW	2	0	13	0	15	10	0	14	0	24
WAY	2	1	10	0	15	6	1	18	0	25

Table 2. Correlation between percentage cover exotic ascidians observed in FTD and UTD surveys.

Taxa	a. Correlation		b. Probability	
	Kendall τ	Pearson r	Kendall test	Pearson test
B. violaceus	0.70	0.77	.017	.014
B. schlosseri	1.00	1.00	.010	<.001
D. listerianum	0.70	0.88	.029	.002
S. clava	1.00	1.00	.029	<.001
Total	0.66	0.87	.014	.001

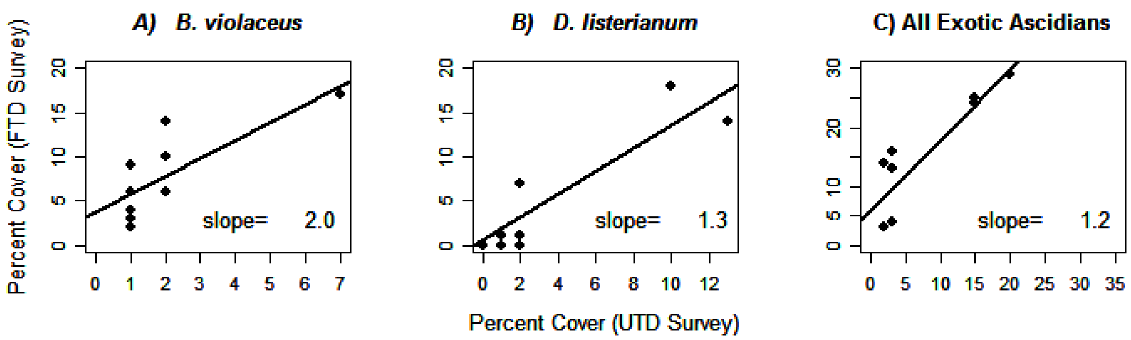

Figure 2. A linear regression of the mean percentage cover at each site estimated by the FTD versus UTD data for A) *Botrylloides violaceus*, B) *Diplosoma listerianum* and C) all exotic ascidians including *Botryllus schlosseri*, *Botrylloides violaceus*, *Diplosoma listerianum*, and *Styela clava*. Means were calculated from 8 samples for FTD method and 12 samples for UTD method.

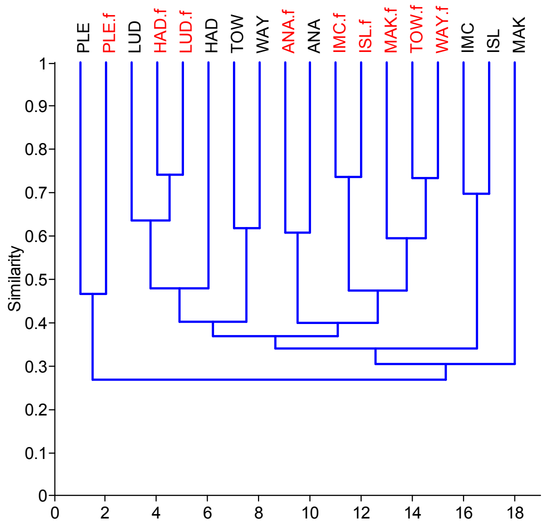

Figure 3. Dendrogram representing the un-weighted paired group average (UPGMA) clustering of Bray-Curtis dissimilarities for FTD and UTD species abundance data. The UTD data for each site is represented by the site code, whereas the FTD method is represented by the site code followed by a lower-case f (e.g. PLE = Pleasant Harbor UTD method, PLE.f = Pleasant Harbor FTD method). Cophenetic Correlation = 0.79.

cover of exotic ascidian species. Unexpectedly, the FTD method generally estimated greater exotic ascidian cover, suggesting that these species may be more abundant on the sides of floating docks. This could indicate a real pattern of exotic species performing better in more sunlit areas (either due to lack of competition, reduced predation or a preference for light), or could simply be a result of systematic observational errors. Further study with increased sampling would be required to distinguish between these two factors.

Both survey methods identified similar, but not completely identical, community patterns, as evidenced by the cluster analysis and Mantel tests. The dendrogram suggests some correspondence of the two survey methods, as the two communities observed by each method in one site often group near each other. There are, however, many exceptions (see MAK, TOW, WAY in Figure 3) and some evident clustering of communities by method (see the FTD cluster on the right side of the tree). That sites clustered together even slightly is unexpected based on previous findings highlighting a significant effect of sunlight exposure on fouling communities and ascidians (Bingham and Reyns 1999, Glasby 1999). One explanation could be that across the regional scale covered in this study other environmental variables such as temperature, salinity and water quality override within-site variation in sunlight exposure. Again, further experimental studies would be needed to tease apart these factors. Mantel tests showed significant correlations between community dissimilarity matrices for 3 of the 4 distance metrics used. This suggests that, while each method observes a different native community, the two methods generate similar among-site patterns. In other words, they detect similar differences in species abundance patterns among sites in this region.

In conclusion, this study demonstrates that surveys of exotic ascidians and, to some extent, fouling communities in marinas can be as effective when carried out from the side of floating docks as underneath them. Of course, more comprehensive surveys covering all substrates and depths would be needed to determine absolutely the presence of exotic species in these habitats. One caveat is that the results of this comparison may depend upon the exotic species surveyed. Other species not present in this study may display different distributional patterns that could alter the correlation between these survey methods. Thus caution and good judgment should be applied when using these methods in other regions. Nonetheless, the survey methods outlined here would be extremely useful for tracking exotic ascidian populations through time and space as well as for comparative ecological studies.

Of the two methods employed in this study, the FTD survey method is easier, less expensive and generally finds the largest abundances of exotic ascidians, so would be more likely to detect species at low abundances. Some drawbacks to this method include potential observer bias if more than one person conducts the surveys and the fact that this method would be inappropriate for marinas where docksides are periodically cleaned. The UTD method is considerably more costly and time-consuming, but it would provide photographs that could be re-analyzed by others or sent to specialists to verify new or unknown exotic ascidians. Overall, the FTD survey would be the best option for rapid, early detection survey programmes while the UTD survey method would be preferable when many people are involved in monitoring or permanent records of sampled plots are required.

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Annex 1

Survey sites examined during this study along the Strait of Juan de Fuca and Upper Puget Sound of Washington, USA.

Code	Site	Location	Coordinates		Date
			Latitude	Longitude	
ANA	Cap Santo Marina	Anacortes	48°30'54" N	122°34'06" W	19.09.2005
HAD	Port Hadlock Marina	Port Hadlock	48°01'54" N	122°44'43" W	17.09.2005
IMC	Island Marine Center	Lopez	48°30'55" N	122°54'56" W	19.09.2005
ISL	Islander Marina	Lopez	48°29'78" N	122°54'02" W	19.09.2005
LUD	Port Ludlow Marina	Port Ludlow	47°55'30" N	122°41'10" W	15.09.2005
MAK	Makah Marina	Neah Bay	48°22'05" N	124°36'42" W	16.09.2005
PAH	Port Angeles Boat Haven	Port Angeles	48°07'38" N	123°27'09" W	18.09.2005
PLE	Pleasant Harbor Marina	Brinnon	47°39'42" N	122°54'56" W	14.09.2005
TOW	Port Townsend Boat Haven	Port Townsend	48°63'89" N	122°46'26" W	17.09.2005
WAY	John Wayne Marina	Sequim Bay	48°03'43" N	123°02'18" W	18.09.2005