

Research Article

Spatial and temporal patterns of native and invasive ascidian assemblages in a Southern California embayment

Brianna M. Tracy and Nathalie B. Reynes*

Environmental and Ocean Sciences, University of San Diego, San Diego, CA 92110, USA

E-mail: briannamtracy@sandiego.edu (BMT), nreyns@sandiego.edu (NBR)

*Corresponding author

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Abstract

Community development of ascidians was monitored weekly in Mission Bay (San Diego, California, USA) from September 2011 through November 2012 to assess how ascidian communities might be structured by spatial differences in environmental conditions. We repeatedly photographed PVC plates that were deployed on floating docks at four sampling locations: Seaforth Landing, Mission Bay Yacht Club, South Shores, and Hilton. Environmental variables (temperature, salinity, visibility, flow and dissolved oxygen) were measured during each sampling period. Eleven of the fifteen ascidian species observed on plates were invasive, three were native, and one was cryptogenic. Ascidian community composition varied significantly by time and sampling location within Mission Bay. Spatial differences in community dissimilarity were correlated to visibility, salinity, and temperature. Ascidian communities established in the warm, hypersaline waters furthest from the mouth of Mission Bay were typically most different from those near the mouth. Comparisons of % dissimilarity at the beginning and end of sampling indicated that ascidian communities were more dissimilar at the end of sampling. Ascidians persisted year round, and invasive and cryptogenic species were generally more abundant in winter months than native species. Below average rainfall, and the consequent relatively warm sea surface temperature and high salinity in a relatively mild climate region like San Diego, probably prevented the winter ascidian die-offs that typically occur in this area. Such environmental changes may be advantageous to invasive ascidians, not only with regard to mediating their persistence, but also in terms of facilitating the establishment, spread, and dominance of future invaders.

Key words: tunicates, environmental conditions, estuary, fouling communities, invasion

Introduction

There has been a rapid increase of marine invasive species within fouling communities, many of which have been introduced via commercial and recreational ship hulls and ballast water (Cohen and Carlton 1995; Ruiz et al. 2000; Wasson et al. 2001; Bullard et al. 2007; Darbyson et al. 2009). Urbanization of coastlines and estuaries, and in particular the pilings and floating docks associated with marinas developed to support commercial shipping and recreational boating, provide habitat for a variety of epibiotic marine organisms that make up fouling communities (Connell 2001). Invasive species are hypothesized to be better-adapted to surviving on such artificial substrates, and may therefore out-compete native species for resources (Byers 2002), resulting in a restructuring of the established food web (Levine 2000; Byers 2002). Aquatic invasive species are known to

change community structure through the local elimination of native species and are among the greatest threats to marine ecosystems (Ruiz et al. 1997, 2000; Stachowicz et al. 1999, 2002; Grosholz et al. 2000; Carlton 2001; Ranasinghe et al. 2005).

Many fouling communities are dominated by invasive ascidian species (Lambert and Lambert 1998, 2003; Ranasinghe et al. 2005). Ascidians are important indicators of invasions due to their short-lived, low-dispersal larval stage; thus, the appearance of an ascidian species in an area previously uninhabited by that species indicates a method of transport that is not naturally occurring (e.g. ballast water or hull fouling; Lambert 2007). Once settled, invasive ascidians are of concern because they often act as strong spatial competitors, frequently displacing native ascidian species, anemones, mussels, algae and other fouling community organisms (Lambert 1968; Olson 1985; Bullard et al. 2007; Grey 2009; Lindeyer and Gittenberger 2011).

Previous ascidian assessments on the United States west coast have primarily focused in the San Francisco Bay area, with comparatively few studies conducted in southern California (Lambert and Lambert 1998; Grosholz et al. 2000; Ranasinghe et al. 2005; Wasson et al. 2005). The first large-scale assessment of southern California harbors was conducted in the early 20th century, and observed ascidians were predominately indigenous, though a few invasive species were noted on dock pilings (Ritter and Forsyth 1917). Although most of the native species identified by Ritter and Forsyth (1917) remain abundant in southern California, they are more prevalent in coastal regions outside of harbors and bays, indicating large temporal and spatial changes in the ascidian communities of these regions in the last century (Fay and Johnson 1971; Lambert and Lambert 1998, 2003). Fourteen invasive ascidian species are now established within southern California (Lambert and Lambert 1998, 2003). It remains unclear, however, if these species reside in fouling communities year-round, and if invasive ascidians settle at a more frequent rate, grow more quickly, and have lower mortality rates, than indigenous ascidians. To our knowledge, no high-resolution temporal studies have quantified ascidian community development in southern Californian (but see Lambert and Lambert 1998, 2003; DeRivera et al. 2005). To begin addressing these questions, the overall objective of this study was to characterize the spatial and temporal patterns of ascidian community development within fouling communities in Mission Bay, San Diego, California, a highly urbanized estuary influenced by varying environmental conditions. Specifically, we hypothesized that ascidian communities would be structured by spatial differences in salinity, temperature, tidal flushing, and water column visibility.

Ascidian communities inhabit regions protected from strong wave action and in environments with salinity of 25 or above (Lambert 2007). Typically, ascidians experience annual cycles as many species die-off in winter months during periods of high freshwater input and decreased temperature, although salinity and temperature tolerances are highly variable and species-specific (Lambert and Lambert 1998, 2003; Tyrrell and Byers 2007). With concern that warming sea surface temperatures due to global climate change could allow ascidian communities to persist perennially, and consequently promote the range expansion of invasive species (Lambert and Lambert 1998,

2003; Lambert 2007; Tyrell and Byers 2007; Darbyson et al. 2009), this study represents a step towards understanding how local environmental conditions influence ascidian community composition and development.

Methods

Study area and sampling locations

Marine fouling communities were monitored within Mission Bay, San Diego, California, USA ($32^{\circ}46'44''$ N, $117^{\circ}13'48''$ W; Figure 1), a shallow mesotidal estuary surrounded by a heavily populated urban area and subjected to multiple sources of environmental degradation (Largier et al. 1997; Kaufmann et al. 2004). Environmental conditions near the mouth of Mission Bay resemble that of a typical coastal ocean environment due to tidal exchange, and become less neritic with increasing distance away from the mouth (e.g., moving upstream; Largier et al. 1997; Kaufmann et al. 2004). Further from the mouth, evaporation and solar heating combined with poor tidal exchange and shallow water depths cause the “back bay” region (Figure 1) of Mission Bay to exhibit warm, hypersaline surface water during the summer months (Largier et al. 1997; Kaufmann et al. 2004). In the winter, periodic rainfall and input from creeks and storm drains cause surface waters to cool and freshen relative to summer temperatures and salinities (Largier et al. 1997; Kaufmann et al. 2004).

Four sampling sites were chosen to (1) represent this range of environmental conditions potentially experienced by ascidians; and (2) incorporate three previously sampled sites in Mission Bay: Seaforth Landing (closest to mouth of Mission Bay; sampled by Lambert and Lambert 1998, 2003), Mission Bay Yacht Club (sampled by Lambert and Lambert 2003) and South Shores (sampled by Lambert and Lambert 2003), and Hilton (within the back bay region; Figure 1). This is the first study, however, to simultaneously examine multiple sites at a relatively high temporal (weekly) sampling resolution in this region.

Sampling plates

At each sampling site, five 13×13 cm plates were simultaneously deployed in September 2011. Plates were cut from grey high-impact strength polyvinyl chloride (PVC) sheets, sanded to optimize attachment conditions for organisms, and seasoned in laboratory seawater tanks with salinity of 35 for one month prior to deployment. After seasoning,

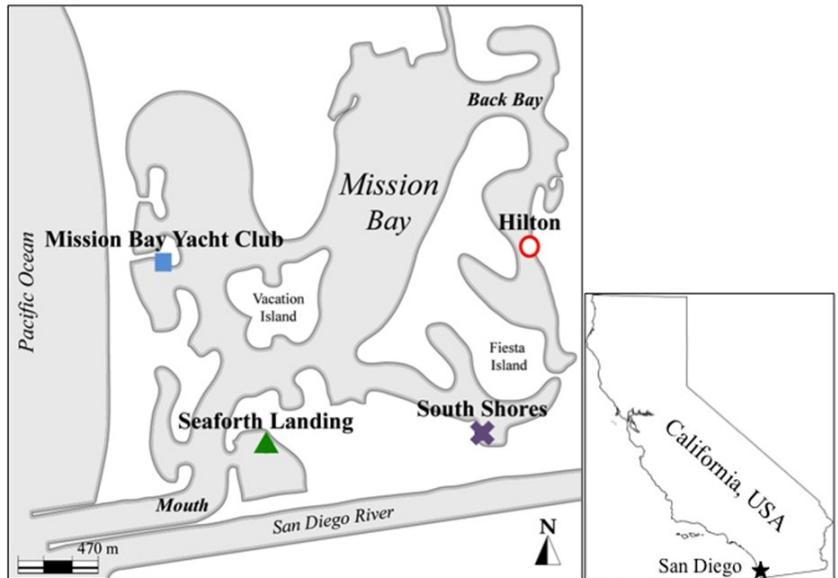


Figure 1. Sampling locations within Mission Bay, San Diego, California, USA. Green triangle represents Seaforth Landing; purple cross represents South Shores; blue square represents Mission Bay Yacht Club; red circle represents Hilton.

plates were attached to the sides of floating docks with cable ties affixed to stainless steel eyebolts. A small stainless steel weight (~60g) was attached to the bottom of each plate to ensure that they hung vertically in the water column to best mimic the sides of floating docks (Tracy 2013). Floating docks were ideal locations for plate deployment as they eliminated the need to document tidal ranges on a daily basis and the threat of organism desiccation. Plates served as passive collectors for the colonization of marine fouling organisms and provided a standardized method to assess the presence and identity of ascidians. Some plates were lost during the study (likely dislodged by boats or removed by anglers or tourists). Although such plates were immediately replaced, fouling community development on clean surfaces did not follow that of the previously established plates. Thus, data from replacement plates were not used in our analysis. At the end of the study, four plates remained at Seaforth Landing and South Shores, respectively, and two plates remained at both Mission Bay Yacht Club and Hilton sampling locations.

Environmental conditions

Environmental variables were recorded at each study location during each sampling date. Temperature, conductivity, and dissolved oxygen were measured using a YSI Model 85 meter (YSI

Incorporated, Yellow Springs, Ohio, USA). A 0.30 m Secchi disk was used to estimate visibility (a measure of turbidity) of the water column.

Clod cards, which provide relative measures of water motion (Jokiel and Morrissey 1993), were deployed at each site. Clods were prepared by using a 1:4 latex paint to plaster of Paris mixture that was molded in ice cube trays. Two clods were glued with marine epoxy to a 13 × 13 cm PVC plate and allowed to dry for one week. Each clod card apparatus was weighed (initial weight) and deployed for the corresponding ascidian sampling interval (one week) via attachment to the docks in the same manner that fouling community plates were deployed. For each sampling interval, percent clod card loss was calculated by subtracting the post-deployment weight of the clod card apparatus after drying for a two-week minimum in the laboratory, from the initial weight of the clod card apparatus before submersion in the field.

Identification of fouling organisms

Ascidians that settled on the plates were identified to species and classified by status of origin as native, invasive, or cryptogenic using past ascidian surveys conducted in southern California (Ritter and Forsyth 1917; Fay and Johnson 1971; Lambert and Lambert 1998, 2003), and discussions with an ascidian taxonomist (Gretchen Lambert, University of Washington Friday Harbor Laboratories,

pers. comm.). Non-ascidians were identified to the lowest taxonomic group possible and generally not classified as invasive or native. For the purpose of this paper, ‘invasive’ is defined as a species that occurs outside its known native region and has established a population in a previously uninhabited area to the detriment of the native community (Carlton 2001; Blackburn et al. 2011), and ‘cryptogenic’ is defined as a species suspected as invasive but with insufficient evidence to confirm its origin (Fofonoff et al. 2003).

Plate photo analysis

To identify and quantify the ascidians, the sampling plates were repeatedly photographed with an Olympus Stylus Tough 8010 14MP Digital Camera. Each week, plates were carefully removed from the water, photographed, and returned to the water so that the ascidian community could be tracked over time. In addition to photographs, descriptions of the fouling communities and ascidian species on the plates were recorded in a field journal at the time the photos were taken. To determine the community composition on each plate, an organism ‘Point Count’ was completed using the image-processing program ImageJ (Abramoff et al. 2004). A 10 cm × 10 cm digital grid was overlaid on each plate image such that individuals were identified to lowest taxonomic level possible on 100 points per plate, and percent cover of each species (number of points occupied by a particular species) was calculated. We referred to field notes to confirm species identifications.

Data analysis

To examine ascidian community structure at sampling locations within Mission Bay, we used a multivariate approach (PRIMER 6.1.10; Clarke and Gorley 2006). The similarity of ascidian community composition at each site (for duration of study: September 2011 – November 2012), was visually assessed using hierarchical clustering and non-metric multi-dimensional scaling (MDS) based on a Bray-Curtis similarity matrix (Bray and Curtis 1957; Clarke and Warwick 2001). We used a similar approach to determine if ascidian community composition differed over time. Given the relatively high number of weeks sampled, however, it was not computationally possible to examine differences from week to week. Thus, we used hierarchical clustering and MDS to evaluate the similarity of ascidian communities at the

beginning (month of November 2011), middle (month of April 2012), end (month of October 2012), and entire duration (September 2011 – November 2012) of the study period. Permutational multivariate analysis of variance (PERMANOVA+ for PRIMER; Anderson et al. 2008) was used to test the null hypothesis of no difference in ascidian community assemblage between the four sampling sites (fixed factor), replicate plates (nested within sites), and time (beginning, middle, end).

PERMANOVA+ results indicated a significant difference in ascidian communities by site and time; thus, multiple analysis of similarities (ANOSIMs; Clarke 1993) were performed to determine how ascidian community composition differed between sampling sites at the beginning, middle, end, and over the entire sampling interval (as defined above). The ANOSIM routine outputs a pairwise R-value ranging from 0 to 1 as an absolute measure of the degree of separation between samples. If R-values are significant ($P < 0.05$), then an R-value near zero indicates no biologically meaningful difference in ascidian species community composition between sampling sites, sites with $R \leq 0.5$ are similar, sites with R-values between 0.5 and 0.75 have overlapping ascidian species but are not the same, and sites with $R \geq 0.75$ are well-separated from one another (*i.e.* not similar; Clarke and Gorley 2006).

The similarity percentage procedure (SIMPER) was used to calculate the percent dissimilarity of ascidian community composition between sites on plates deployed during the beginning, middle, end, and entirety of the study (as defined above), where 0% indicates no difference and 100% maximum difference. SIMPER was also used to determine which ascidian species were primary contributors to the differences in fouling community at each sampling location during the study period.

To determine if sampling sites could be distinguished by differences in environmental variables (water temperature, salinity, flow, and visibility), the variables were log+1 transformed, normalized, and a dissimilarity matrix was calculated from Euclidean distance. The BIO-ENV procedure was used to identify the combination of environmental variables that best explained ascidian community patterns (Spearman rank correlation ρ_w , Clarke and Ainsworth 1993) at our sampling sites. Dissolved oxygen varied slightly by site but remained within a range (6.1–10.9 mg/L; Tracy 2013) that is not of environmental concern (Stoddard et al. 2006), and was therefore not included in the analysis.

Table 1. Average \pm standard deviation and range (below in parentheses) of environmental variables measured at Mission Bay sampling sites from September 2011 – November 2012. *relatively shallow (2.5m depth) bottom was always visible.

Sampling Sites	Water Temperature (°C)	Salinity	Flow (% Clod Card Loss)	Visibility (Secchi Disk Depth in m)
Seaforth Landing	17.15 \pm 2.32 (11.2–17.6)	34.14 \pm 0.43 (32.7–34.3)	52.2 \pm 10.93 (32.0–78.2)	6.87 \pm 0.48 (4.8–8.0)
South Shores	18.45 \pm 2.98 (12.3–23.2)	33.96 \pm 1.04 (30.1–34.6)	34.0 \pm 7.98 (14.1–53.4)	5.30 \pm 0.64 (3.9–6.1)
Mission Bay Yacht Club	17.47 \pm 2.44 (13.8–21.6)	34.11 \pm 0.66 (30.1–35.0)	45.0 \pm 14.51 (18.0–90.2)	>2.5* (2.5)
Hilton	18.69 \pm 3.42 (11.9–23.7)	33.06 \pm 5.10 (5.2–35.6)	24.4 \pm 4.94 (10.4–50.2)	2.43 \pm 0.57 (2.0–3.2)

Results

Environmental conditions

Seaforth Landing had the lowest average and smallest range of water temperature, the highest average salinity, as well as the highest flow and Secchi depth of all sampling sites (Table 1). In contrast, Hilton had the highest average and largest range of water temperature, the lowest average salinity, largest range of salinity, the lowest average flow of all sampling sites, and relatively poor water column visibility. Both South Shores and Mission Bay Yacht Club had intermediate environmental conditions. At Mission Bay Yacht Club, it was always possible to see the bottom due to the shallow (2.5 m) depth of this site in comparison to the depths of the other sampling sites (> 5 m).

Hierarchical clustering and MDS ordination of all environmental variables measured at each Mission Bay sampling site depicted some clustering between locations (sites appeared to have similar environmental variables), with Hilton having four distinguishable decreases in salinity that occurred during rain events in San Diego (Figure 2A). The BIO-ENV procedure indicated that variation in ascidian community composition was best correlated to one environmental variable: visibility ($\rho_w = 0.370$, Table 2; Figure 2B). Correlation coefficients were similar for combinations that included ‘salinity-visibility’ ($\rho_w = 0.350$; Figure 2C), ‘visibility-water temperature’ ($\rho_w = 0.345$; Figure 2D; note relatively stable temperatures and visibility at each site resulted in linear pattern), and ‘salinity-visibility-water temperature’ ($\rho_w = 0.327$; Table 2; Figure 2E).

Table 2. BIO-ENV combinations of the four environmental variables, taken k at a time, producing the best matches of similarity matrices, as measured by weighted Spearman rank correlation ρ_w .

Number of Variables (k)	Best Variable Combinations	Correlation (ρ_w)
1	Visibility	0.370
2	Salinity-Visibility	0.350
2	Visibility-Water Temperature	0.345
3	Salinity-Visibility-Water Temperature	0.327
3	Flow-Visibility-Water Temperature	0.309
4	Flow-Salinity-Visibility-Water Temperature	0.297
2	Flow-Visibility	0.283
3	Flow-Salinity-Visibility	0.272
2	Flow-Water Temperature	0.163
3	Flow-Salinity-Water Temperature	0.162

Ascidian communities

Fifteen ascidian species were observed on plates throughout the duration of this study: *Ascidia zara* Oka, 1935, *Botrylloides violaceus* Oka, 1927, *Botryllus schlosseri* (Pallas, 1766), *Ciona intestinalis* (Linnaeus, 1767), *Ciona savignyi* Herdman, 1882, *Didemnum vexillum* Kott, 2002, *Molgula ficus* (Macdonald, 1859), *Polyandrocarpa zorritensis* (Van Name, 1931), *Styela clava* Herdman, 1881, *Styela plicata* (Lesueur, 1823), *Symplegma reptans* (Oka, 1927), *Aplidium californicum* (Ritter and Forsyth, 1917), *Ascidia ceratodes* (Huntsman, 1912), *Botrylloides diegensis* Ritter and Forsyth, 1917, and *Diplosoma listerianum* (Milne-Edwards, 1841) (Table 3). Seaforth Landing and South Shores had the greatest number of

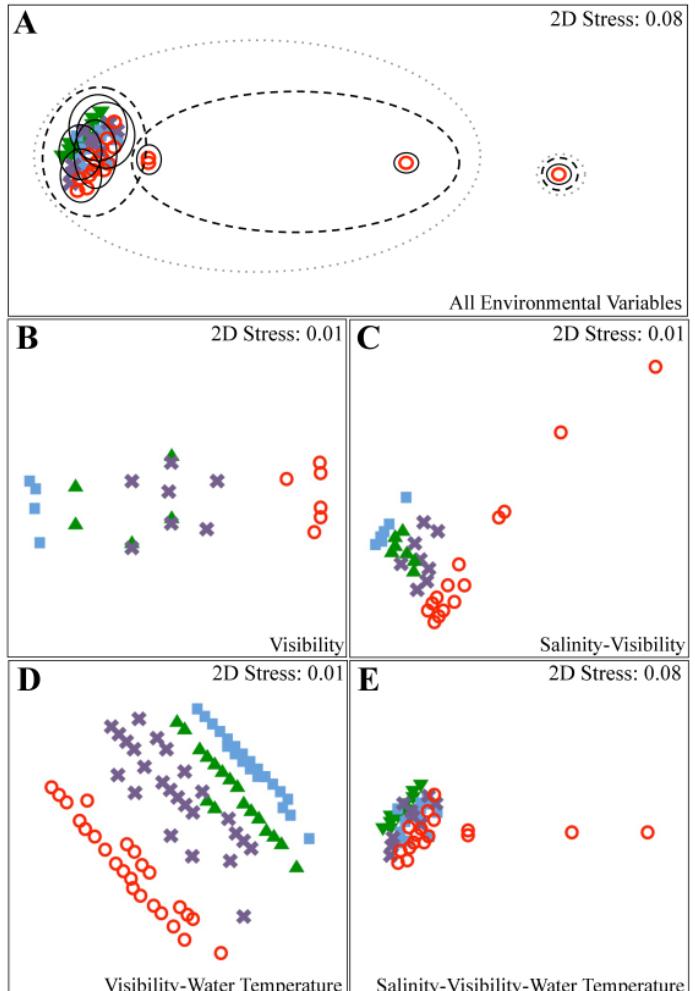


Figure 2. Non-metric Multi-Dimensional ordinations of Euclidean distance dissimilarity matrices of environmental variables at Mission Bay sampling locations from September 2011 through November 2012. Green triangle represents Seaforth Landing; purple cross represents South Shores; blue square represents Mission Bay Yacht Club; red circle represents Hilton. Contours indicate levels of similarity between sampling locations (solid-line black = 5%; dashed-black line = 10%; dotted-grey line = 15%). All combined environmental variables (water temperature, salinity, flow, visibility) in panel (A); Subsets of environmental variables best correlated with ascidian community structure (Spearman rank correlation $\rho_s > 0.327$; see Table 2): (B) Visibility; (C) Salinity and visibility; (D) Visibility and water temperature; (E) Salinity, visibility and water temperature.

ascidian species with thirteen; Mission Bay Yacht Club had eleven species; and Hilton had only three species.

Of ascidians identified in Mission Bay, 73.3% (11 of 15 species) were invasive species (Table 3), and no native ascidian species dominated the ascidian communities at any of the four sampling locations. *Ciona intestinalis* accounted for over 24% of the ascidian community at all locations except for Mission Bay Yacht Club where it accounted for less than 3% of the space occupancy. At Mission Bay Yacht Club, the dominant ascidian species was *B. violaceus*, accounting for 57.5% of the space occupancy. South Shores was the only sampling location where ascidian species *D. vexillum* and *M. ficus* were found. Hierarchical clustering and MDS ordination of ascidian community composition at each sampling site

confirmed clustering within a given sampling site and dissimilarity between sampling locations (Figure 3A).

Plates were deployed for approximately one month before it was possible to visually identify settled ascidians in the field without a microscope. By November 2011, plates were colonized by invasive ascidians *A. zara*, *B. violaceus*, *B. schlosseri*, *C. intestinalis*, *D. vexillum*, *S. clava*, and *S. plicata*, native species *A. californicum* and *B. diegensis*, and the cryptogenic species *D. listerianum* (Table 4). Invasive ascidians were the most abundant species throughout the duration of the study, with greatest abundances from December through May 2012: *B. violaceus*, *B. schlosseri*, and *C. intestinalis*. Abundances of *D. listerianum* also remained relatively high during this period. *C. intestinalis* continued to occupy space at

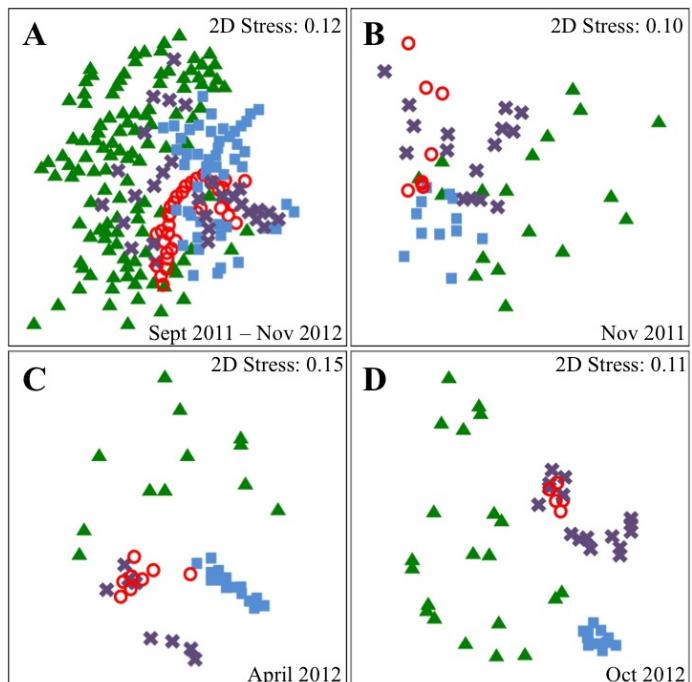


Figure 3. Non-metric Multi-Dimensional ordination of Bray-Curtis similarities of ascidian community composition at Mission Bay sampling locations on plates deployed from September 2011 through November 2012 (A), and examined at the beginning (month of Nov. 2011; Seaford Landing n = 5 plates, South Shores n = 5, Mission Bay Yacht Club n = 5, Hilton n = 5; B), middle (month of April 2012; Seaford Landing n = 4, South Shores n = 5, Mission Bay Yacht Club n = 5, Hilton n = 4; C), and end (month of Oct. 2012; Seaford Landing n = 4, South Shores n = 4, Mission Bay Yacht Club n = 2, Hilton n = 2; D) of the sampling interval. Green triangle represents Seaford Landing; purple cross represents South Shores; blue square represents Mission Bay Yacht Club; red circle represents Hilton.

Table 3. Average percent cover during the entire sampling interval (September 2011 – November 2012) of invasive, native, and cryptogenic ascidian species on Mission Bay plates by sampling site.

Species	Seaford Landing	South Shores	Mission Bay Yacht Club	Hilton
Invasive				
<i>Ascidia zara</i> Oka, 1935	0.36	0.23	0.56	-
<i>Botrylloides violaceus</i> Oka, 1927	8.13	0.03	57.48	-
<i>Botryllus schlosseri</i> (Pallas, 1766)	16.01	30.02	5.04	-
<i>Ciona intestinalis</i> (Linnaeus, 1767)	24.69	24.74	2.78	84.49
<i>Ciona savignyi</i> Herdman, 1882	3.68	1.53	0.15	-
<i>Didemnum vexillum</i> Kott, 2002	-	12.53	-	-
<i>Molgula ficus</i> (Macdonald, 1859)	-	1.82	-	-
<i>Polyandrocarpa zorritensis</i> (Van Name, 1931)	0.88	17.09	7.78	-
<i>Styela clava</i> Herdman, 1881	3.81	0.32	0.19	4.02
<i>Styela plicata</i> (Lesueur, 1823)	2.92	7.56	-	-
<i>Symplema reptans</i> (Oka, 1927)	6.89	-	12.96	-
Native				
<i>Aplidium californicum</i> (Ritter and Forsyth, 1917)	3.55	3.61	-	11.49
<i>Ascidia ceratodes</i> (Huntsman, 1912)	0.05	-	0.15	-
<i>Botrylloides diegensis</i> Ritter and Forsyth, 1917	7.53	0.03	12.48	-
Cryptogenic				
<i>Diplosoma listerianum</i> (Milne-Edwards, 1841)	21.51	0.49	0.44	-

a relatively high rate into August 2012. *Molgula ficus* was not present in Mission Bay at any sampling location until April 2012 and *P. zorritensis* did not establish until August 2012. *Botrylloides violaceus*, *B. schlosseri*, *C. intestinalis*, *D. vexillum*, *S. plicata*, *B. diegensis*, and *D. listerianum* were present every month for the duration of the study in varying abundances.

Spatial and temporal patterns

The factors site, time, and plate all had significant effects on ascidian community composition (Table 5). There were also significant interactions when site × time and plate × time were combined. Hierarchical clustering and MDS ordination of

Table 4. Abundance and monthly occurrence of ascidians in Mission Bay from November 2011 – October 2012 based on total ascidian abundance at all sampling locations combined. (+): species present in low numbers (<10% coverage); (++) commonly found (11-50%); (+++): abundant (51-84%); (++++): high species coverage on plates (>85% coverage).

Ascidian species	Sampling month											
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
Invasive												
<i>Ascidia zara</i>	+	+	-	+	-	+	+	+	+	+	+	+
<i>Botrylloides violaceus</i>	+	++	+++	+++	+++	+++	++	+	+	+	+	+
<i>Botryllus schlosseri</i>	++	+++	+	++	++	++	++	+	+	+	+	+
<i>Ciona intestinalis</i>	+	+	++	++	++	++	+++	+++	++	+	+	+
<i>Ciona savignyi</i>	-	+	+	+	-	+	+	+	+	+	+	+
<i>Didemnum vexillum</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Molgula fucus</i>	-	-	-	-	-	+	+	+	+	+	+	+
<i>Polyandrocarpa zorritensis</i>	-	-	-	-	-	-	-	-	-	+	+	++
<i>Styela clava</i>	+	+	+	+	+	+	+	+	+	-	-	-
<i>Styela plicata</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Symplema reptans</i>	-	-	-	-	+	+	+	+	+	++	++	+
Native												
<i>Aplidium californicum</i>	+	+	+	+	+	+	-	+	+	+	+	+
<i>Ascidia ceratodes</i>	-	-	+	-	-	-	-	+	+	-	-	-
<i>Botrylloides diegensis</i>	+	+	+	+	+	+	+	+	+	++	++	++
Cryptogenic												
<i>Diplosoma listerianum</i>	+	+	++	++	++	+	++	+	+	+	+	+

Table 5. PERMANOVA+ analysis of the effects of sampling sites (fixed), replicate plates (nested within sites), and time on ascidian communities at Mission Bay sampling sites; p-values were obtained using 999 permutations.

Source of Variation	df	P
Site	3	0.001
Time	3	0.001
Plate (Site)	15	0.001
Site × Time	6	0.002
Plate (Site) × Time	20	0.001

ascidian community composition at each sampling site visually confirmed this dissimilarity between sites over time (compare Figures 3B-D), as well as amongst replicate plates at each site (see clustering of symbols in Figures 3B-D). Our focus, however, was to further examine the between-site differences in ascidian communities rather than within-site differences, given that we recorded environmental conditions at each site and not at each plate.

In terms of spatial differences in ascidian communities, multiple analyses of similarities (ANOSIMs) indicated varying degrees of similarity when sampling sites were compared to one-another (Table 6). Seaforth Landing, South Shores, and Hilton exhibited similar ascidian community composition when considering the entire sampling period ($R \leq 0.5$; $P < 0.001$). Ascidian communities at Seaforth Landing compared to Mission Bay

Yacht Club and South Shores compared to Mission Bay Yacht Club, however, had overlapping species, but were considered different ($R = 0.5$ –0.75; $P < 0.001$). The ascidian communities at Mission Bay Yacht Club and Hilton had the highest R-value of all site-comparisons, indicating differences in species composition ($R = 0.730$; $P < 0.001$).

Ascidian community composition changed between the beginning (month of November 2011), middle (month of April 2012), and end (month of October 2012) periods of our study. Seaforth Landing and South Shores were progressively more dissimilar as sampling proceeded (Table 6). In contrast, Seaforth Landing and Mission Bay Yacht Club, and South Shores and Mission Bay Yacht Club exhibited comparable trends, whereby communities were least different in November ($R \leq 0.5$; $P < 0.01$), diverged in April 2012 ($R > 0.7$; $P < 0.001$), but became more similar toward October 2012 ($R \leq 0.5$; $P < 0.001$). Although P-values for comparisons in the middle and end were not statistically significant, South Shores and Hilton, had some overlapping ascidian communities at the beginning. Differences between Seaforth Landing and Hilton were more pronounced, as ascidian communities were well-separated at the beginning ($R = 0.795$; $P = 0.004$), became more similar in April ($R \leq 0.5$; $P = 0.007$), and diverged at the end ($R = 0.994$; $P = 0.042$) of the sampling period (Figure 3B-D).

Table 6. Analysis of similarity (ANOSIM) of ascidian species community composition compared between locations at the beginning (month of November 2011), middle (month of April 2012), end (month of October 2012), and during the entirety of the sampling period (September 2011–November 2012). R-values are indicated with p-values in parentheses below. *indicates a significant difference between sites.

	Beginning	Middle	End	Entire Interval
Mission Bay Sampling Sites	(Nov. 2011)	(Apr. 2012)	(Oct. 2012)	(Sept. 2011-Nov. 2012)
Seaforth Landing, South Shores	0.183 (0.019*)	0.342 (0.002*)	0.696 (<0.001*)	0.299 (<0.001*)
Seaforth Landing, Mission Bay Yacht Club	0.430 (<0.001*)	0.719 (<0.001*)	0.458 (<0.001*)	0.528 (<0.001*)
Seaforth Landing, Hilton	0.795 (0.004*)	0.432 (0.007*)	0.994 (0.042*)	0.416 (<0.001*)
South Shores, Mission Bay Yacht Club	0.395 (<0.001*)	0.915 (<0.001*)	0.015 (0.373)	0.672 (<0.001*)
South Shores, Hilton	0.559 (0.009*)	0.035 (0.368)	0.654 (0.059)	0.419 (<0.001*)
Mission Bay Yacht Club, Hilton	1 (0.008*)	1 (0.005*)	1 (0.200)	0.730 (<0.001*)

Table 7. SIMPER dissimilarities (%) of ascidian community composition at Mission Bay sampling sites on plates deployed from September 2011 through November 2012 at the beginning (month of November 2011), middle (month of April 2012), end (month of October 2012), and during the entirety of the of the sampling period; 0 indicates no difference, 100 maximum difference.

	Beginning	Middle	End	Entire Interval
Mission Bay Sampling Sites	(Nov. 2011)	(Apr. 2012)	(Oct. 2012)	(Sept. 2011-Nov. 2012)
Seaforth Landing vs. South Shores	5.1	26.0	32.4	24.6
Seaforth Landing vs. Mission Bay Yacht Club	9.5	22.8	4.0	32.2
Seaforth Landing vs. Hilton	62.7	26.4	100.0	41.4
South Shores vs. Mission Bay Yacht Club	13.5	3.4	31.5	30.1
South Shores vs. Hilton	60.3	0.4	100.0	35.4
Mission Bay Yacht Club vs. Hilton	67.9	3.8	100.0	45.1

Table 8. SIMPER contribution (%) of ascidian species at Mission Bay sampling sites listing taxa in the order they contribute to dissimilarity between sampling locations (site) on plates deployed from September 2011 through November 2012.

Site	Ascidian Species	Contribution (%)
Seaforth Landing	<i>Ciona intestinalis</i>	31.46
	<i>Diplosoma listerianum</i>	25.26
	<i>Botryllus schlosseri</i>	23.92
	<i>Botrylloides violaceus</i>	4.33
	<i>Botrylloides diegensis</i>	3.92
	<i>Aplidium californicum</i>	3.90
South Shores	<i>Botryllus schlosseri</i>	57.25
	<i>Didemnum vexillum</i>	13.81
	<i>Ciona intestinalis</i>	11.37
	<i>Styela plicata</i>	8.47
Mission Bay Yacht Club	<i>Botrylloides violaceus</i>	78.84
	<i>Botrylloides diegensis</i>	5.27
	<i>Botryllus schlosseri</i>	5.01
	<i>Symplema reptans</i>	4.95
Hilton	<i>Ciona intestinalis</i>	90.28

Further examination of between-site differences in ascidian communities indicated that Hilton was 60.3–67.9% different from the other sites at the beginning of the study (Table 7). Seaforth Landing was most different from the other locations in the middle of sampling (April 2012: 22.8–26.4% difference), while Hilton was most

different than the other sites by the end of sampling (October 2012: 100% difference). When considering the entire study period (September 2011–November 2012), sites were moderately different from one-another (24.6%–45.1%). These site-specific differences in ascidian communities were also visually apparent (Figure 4).

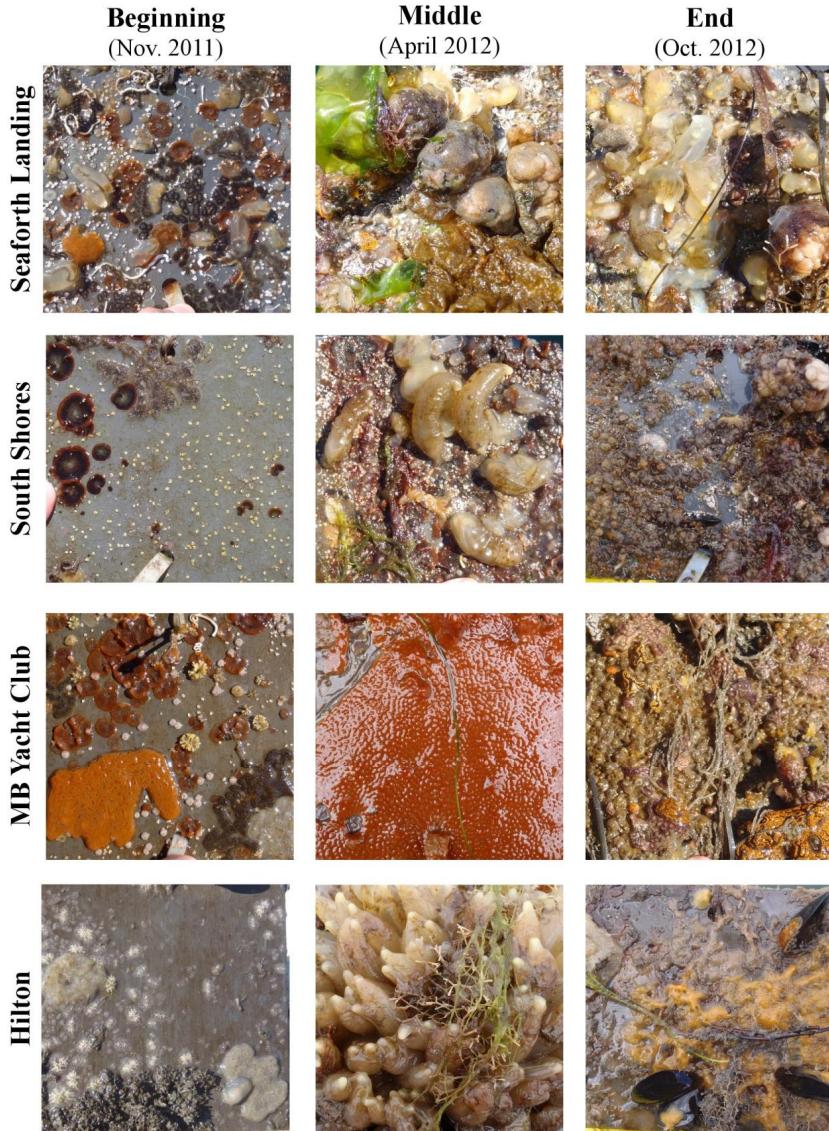


Figure 4. Representative photos used for point counts of ascidian community composition at Mission Bay sampling sites on plates initially deployed September 2011 at the beginning (month of November 2011), middle (month of April 2012), and end (month of October 2012) of the sampling interval. Photographs by B. Tracy.

Species-specific contributions to similarity within sampling sites

While some sites had similar ascidian species on the plates (Table 4), every sampling location was different in terms of the relative contribution of particular species to the dissimilarity between sampling locations (Table 8). For example, *C. intestinalis* contributed to 90.3% of the dissimi-

larity in community composition at the Hilton, whereas *B. violaceus* accounted for 78.8% of the dissimilarity in ascidian community at Mission Bay Yacht Club. Seaforth Landing exhibited the widest variability in ascidian species contribution: *C. intestinalis* explained 31.5% of the dissimilarity in community composition between other sampling location, while *D. listerianum* and *B. schlosseri* explained 25.3% and 23.9% of the dissimilarity, respectively.

Discussion

Spatial and temporal patterns in Mission Bay

Hydrodynamic conditions are known to spatially structure ascidian communities in other systems (Naranjo et al. 1996); however, flushing by currents (flow) in Mission Bay did not explain spatial differences in ascidian communities. While we observed, on average, a trend of decreasing relative flow rates with increasing distance from the mouth of the bay (Seaforth Landing to Hilton), ascidian community development was likely not significantly impacted by the currents because all sites experienced variability in flow.

Twice daily tidal flushing enhances ocean and bay water exchange near the mouth of Mission Bay (Largier et al. 2003). Seaforth Landing, Mission Bay Yacht Club, and South Shores are in close enough proximity to one another that tidal currents (Largier et al. 2003) could allow connectivity via larval dispersal (assuming an average ascidian larval duration of ~24 h; see Fofonoff et al. 2003 for species-specific larval durations). In contrast, flushing is minimal in back-bay regions where water residence times can be relatively long (7–30 days; Largier et al. 2003). Thus, it is possible that recruitment limitation in the back bay site might explain some of the between-site variability in ascidian communities that we observed when comparing Hilton to the other sites, but this was not explicitly tested in this study. Part of the challenge in understanding recruitment limitation is that we know little about the reproductive and spawning patterns of ascidians in Mission Bay.

Ascidian community structure in Mission Bay was most strongly correlated to water column visibility. Visibility varied from week to week (Tracy 2013), but Hilton had the lowest visibility of all sampling sites, potentially indicating sediment input and nutrient loading from Cudahy and Tecolote Creeks located to the north and south, respectively. Sediments in this region are primarily silts (Kaufmann et al. 2004), which can remain suspended in the water longer than the coarser sediments (sand) typically found near the mouth of Mission Bay (Kaufmann et al. 2004). Suspended fine sediments can increase mortality of juvenile ascidians (Young and Chia 1984), clog ascidian siphons (Bakus 1968), and alter epifaunal community development (Maughan 2001). This may explain why Hilton had the lowest ascidian diversity of all our sampling sites. Additionally, Hilton had the highest percent cover of *C. intestinalis* and *S. clava*, both relatively large-bodied solitary

ascidians with large siphons that may resist clogging better than smaller species (Naranjo et al. 1996).

Salinity and temperature were also correlated to ascidian community structure, though adding these explanatory variables to visibility slightly decreased ρ_v , suggesting that salinity and temperature have more minor impacts on ascidian community structure in Mission Bay than visibility (Clarke and Ainsworth 1993) or effects of collinearity of variables. This is likely because all ascidian species identified in this study (with the exception of the native *A. californicum*) were observed within their environmental tolerances for water temperature and salinity (Fofonoff et al. 2003), not including five rainy events (Tracy 2013).

In relatively shallow Southern California estuaries such as Mission Bay, decreasing temperature and salinity occur during winter rain events (Largier et al. 1997). Numerous studies have reported ascidian die-offs and changes in community composition during episodes of decreased temperature and high freshwater input (Lambert and Lambert 1998, 2003, 2007; Tyrrell and Byers 2007). Likewise, during an initial assessment of Mission Bay ascidian communities conducted in early January 2011 (following the ninth wettest San Diego December on record; Western Regional Climate Center 2013), we observed considerable ascidian mortality at South Shores and Mission Bay Yacht Club (BM Tracy and NB Reynolds, pers. obs.). During 2011–2012 sampling, however, there was no large-scale ascidian die-off at any location. The 2011–2012 rainy season (defined as 1 October – 30 April for the study region) had below-average rainfall (Western Regional Climate Center 2013), and episodic decreased surface salinities (e.g., salinity of 5.23 recorded at Hilton on 12 December 2011 during rainy event) were primarily confined to the back-bay site and never persisted for more than several days (Tracy 2013). Fouling communities that do not experience annual or seasonal senescence induced by environmental conditions such as decreased temperature or salinity, and are located in regions with anthropogenic influences and mild climates, may become dominated by invasive species (Tyrrell and Byers 2007).

Invasive ascidians were ubiquitous in Mission Bay, and plate communities resembled those along the docks (BM Tracy, pers. obs.). At all sampling sites, both native and invasive species initially settled on recently deployed plates, but while native ascidians were present throughout the duration of the study, invasive ascidian species dominated the ascidian space occupied on those

plates. As observed in other studies, this suggests that invasive species occupy space at the expense of native ascidian species (Blum et al. 2007; Tyrrell and Byers 2007). Indeed, the invasive species *B. violaceus*, *B. schlosseri*, and *C. intestinalis* were primary contributors to the similarity between plates within sites. The cryptogenic species *D. listerianum* also contributed to the similarity between plates at Seaforth Landing, where this species had relatively high abundances.

Botrylloides violaceus, *B. schlosseri*, and *C. intestinalis* comprised the majority of invasive ascidian species but were not found consistently at every sampling location. This was also true of native species *A. californicum* and *B. diegensis*, and cryptogenic species *D. listerianum*, although they were in considerably lower abundances than the invasive species. *B. diegensis*, a colonial ascidian native to the region (Ritter and Forsyth 1917; Lambert and Lambert 1998), has been reported as a locally dominant space holder when it is an invader outside of its native range (Carlton 1989). In Mission Bay, *B. diegensis* was an early colonizer, and remained present for the duration of this study, but in smaller percent space occupation than the space occupied by invasive species *B. schlosseri*, *B. violaceus*, and *C. intestinalis* (Tracy 2013).

In comparison to previous studies, we also observed differences in the distribution of the invasive species *S. reptans* within Mission Bay. Lambert and Lambert (1998) previously reported that *S. reptans* inhabited the most sheltered regions of Mission Bay furthest from the mouth. In contrast, *S. reptans* was found most frequently at Seaforth Landing and with decreasing frequency away from the mouth of the bay. This shift in *S. reptans* distribution within Mission Bay could be linked to spatial competition with other species, particularly *C. intestinalis*. *Ciona intestinalis* was the dominant species at Hilton (back bay region furthest from the mouth) and has been previously reported to suppress fouling species richness and change community composition (Blum et al. 2007).

When *S. reptans* was first observed in Mission Bay in 1997 (Lambert and Lambert 1998), it was proposed that this species might follow a “boom and bust” pattern characteristic of many introduced species where an initial population explosion almost or completely disappears in a year or two (Williamson 1996). Our study indicates that while the distribution of *S. reptans* within Mission Bay appears to have shifted, this species has successfully colonized the area and persisted during the last fifteen years.

Overall persistence of the invasive species identified in this study may have been mediated by the relative stability (i.e. large fluctuations in temperature or salinity were lacking) of environmental conditions experienced in Mission Bay, especially near the mouth of the bay which experiences exchange with the coastal ocean. This, coupled with rising ocean temperatures due to global climate change may act synergistically to drive invasive species range expansions and persistence (Dukes and Mooney 1999; Stachowicz et al. 2002). Compositional shifts from native to invasive species may occur with an increase in water temperature (Sorte and Stachowicz 2011), due to direct impacts on ascidian spawning and recruitment patterns (Stachowicz et al. 2002), and because some invasive species are more tolerant than native species to such changes (Sorte et al. 2010). Furthermore, changing global temperatures are hypothesized to disproportionately affect communities living within shallow bays and estuaries (Stachowicz et al. 2002), such as Mission Bay. Ultimately, climate-induced changes in the relative abundance of native versus invasive species may result from altered larval dispersal and population connectivity due to changing circulation or shipping (anthropogenic) patterns, as well as altered post-settlement interactions due to physiological stresses from changing thermal tolerances and water chemistry (reviewed by Occhipinti-Ambrogi 2007).

Ascidian assemblages in Mission Bay

The number of replicate plates decreased over the duration of the study due to loss; however, unlike *P*-values that denote statistical significance, *R*-values (ANOSIM) are absolute measures of how separated groups are, and are not affected by the number of replicates used in pairwise comparisons (Clarke and Gorley 2001). For example, a relatively large *R*-value (1; indicating separation between Mission Bay Yacht Club and Hilton) with a relatively large *P*-value (0.2; indicating no statistical difference between sites) in October 2012 is likely due to low replication of plates at Hilton by the end of the study (Clarke and Gorley 2001). Thus, while sites could not be statistically distinguished as different, *R*-values and examination of the photos demonstrate that they were indeed occupied by different ascidian species (see Figure 4).

We observed some general patterns of ascidian succession. Colonial ascidian species *A. californicum*, *B. diegensis*, *B. schlosseri*, *B. violaceus* were the primary colonizers on plates after initial

deployment. These results coincide with a study conducted along the Dutch coast in 2008 and 2009 in which *B. schlosseri* and *B. violaceus* emerged as primary settlers on fouling community plates (Gittenberger and van der Stelt 2011). We also observed that solitary species prevailed later in succession after the initial establishment of the fouling community, and appeared to suppress colonial species in their competition for space (Greene et al. 1983). Succession of colonial species, followed by solitary species, can be explained by the life-history characteristics of the organisms, as the established community shifts towards longer-lived ascidians (solitary species such as *C. intestinalis*) that replace early colonial colonizers (Connell and Slatyer 1977). An exception to these findings was settlement of the colonial species *P. zorritensis* in relatively high abundance during the final three months of sampling. This might indicate that reproductive *P. zorritensis* were not present in Mission Bay until September 2012, or that this species can only settle after the fouling community becomes established.

Once the fouling community became established, both colonial and solitary ascidians settled on various other hard-substrate fouling organisms (e.g., barnacles and bryozoans; Tracy 2013), suggesting that space was a limiting resource as documented in other studies (Dayton 1971; Osman 1977). Ascidians never settled on sponges and rarely settled on other ascidian species (exceptions being *A. californicum* and *S. leptans* which were observed on the outer tunic of *S. clava* and *S. plicata*), likely due to the ability of ascidians to synthesize chemical compounds that inhibit settlement of other individuals (Davis 1991). Ascidians also competed for space with other taxa, including the invasive bryozoan, *Watersipora* sp., which was common at Seaforth Landing, South Shores, and Mission Bay Yacht Club. Another invasive bryozoan, *Zoobotryon verticillatum* (Delle Chiaje, 1822), grew in sizeable mats at Hilton, often completely encasing the plates. While the encrusting bryozoan *Watersipora* sp. could potentially act as a substrate for ascidian attachment (Dean and Hurd 1980), the spaghetti-like branches of *Z. verticillatum* did not provide any additional hard-substrate for the recruitment of ascidians. Thus, plates at Hilton were space-limited during a *Z. verticillatum* population explosion from July–October 2012 (Tracy 2013), likely also contributing to the relatively low species richness observed at this site. Given that ascidian community development

had relatively low correlations with measured environmental conditions (ρ_w range = 0.162–0.370), other factors not explicitly quantified in our study (e.g. biotic interactions such as competition for space, predation, etc.) may be important determinants of community structure. Shading is also known to impact ascidian settlement and survival, and some individuals may be susceptible to UV-radiation (Bingham and Reijns 1999; Glasby 1999). While shading due to surrounding boats, pillars, and other docks might explain some within-site variability, none of our sampling locations were covered by structures such as canopies; therefore, shading did not contribute to between-site differences in ascidian communities.

Several ascidians species were previously identified in Mission Bay ascidian assessments (Lambert and Lambert 2003) that were not present during this study: *Ascidia* sp. Lambert and Lambert 1998, *Botrylloides perspicuum* Herdman, 1886, *Molgula manhattensis* De Kay, 1843, *Microcosmus squamiger* Michaelsen, 1927, *Perophora annexens* Ritter, 1893, and *Styela canopus* Savignyi, 1816. In Fall 1997, *Ascidia* sp. was reported to have decreased in abundance in all areas north of San Diego, but it remained common in San Diego bays and harbors until 2000 (Lambert and Lambert 2003). Lambert and Lambert (2003) hypothesized that *Ascidia* sp. would expand its distribution under favorable environmental conditions (e.g. return of cooler waters following a warming trend due to El Niño), and eventually return to these areas. Our study took place during La Niña/El Niño neutral conditions, but we did not observe *Ascidia* sp. in Mission Bay. Although we quantified the ascidian communities on dock sides weekly, our sampling methods did not attempt to identify potential ascidian refuge populations (e.g. underneath docks, on submerged dock pilings), thus we cannot definitively conclude that *Ascidia* sp. is no longer present in Mission Bay without conducting extensive underwater surveys. An important consideration is that the return of favorable conditions for the reestablishment of *Ascidia* sp. may be unlikely with rising ocean temperatures due to climate change.

Conclusion

In general, some differences in ascidian community composition between sampling locations could be explained by spatial environmental variables, but biotic interactions cannot be discounted. Future studies might also consider experimentally transplanting settlement plates among different locations

within Mission Bay, to better elucidate the roles of environmental variation and recruitment limitation on ascidian community composition. Although no new ascidian invaders were identified during this study, the persistence of invasive species when compared to historic assessments (e.g. Ritter and Forsyth 1917; Fay and Johnson 1971; Lambert and Lambert 1998, 2003) might be indicative of the ability of these species to thrive in mild climate regions, and has implications for understanding how future changes in global climate may disproportionately favor invasive species.

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