

The non-native solitary ascidian *Ciona intestinalis* (L.) depresses species richness

Julia C. Blum^{*,1}, Andrew L. Chang⁵, Marcela Liljesthröm², Michelle E. Schenk³,
Mia K. Steinberg⁴, Gregory M. Ruiz

Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, MD 21037, United States

Received 1 September 2006; received in revised form 1 October 2006; accepted 9 October 2006

Abstract

Non-native ascidians are a dominant feature of many sessile marine communities throughout the world and may have negative effects on species diversity. We tested effects of the non-native *Ciona intestinalis* on the sessile invertebrate community in San Francisco Bay, where it occurs in dense aggregations. In particular, we compared species richness between PVC panels from which *C. intestinalis* were experimentally removed to panels with naturally dense *C. intestinalis* growth, using fouling panels of four sizes (between 49 cm² and 1177 cm²) to measure the effect of *C. intestinalis* recruitment on species-area relationships. We initially deployed 120 fouling panels (30 of each size) at a site known to have dense populations of *C. intestinalis*, assigning these to three different treatments: (1) Experimental removal, whereby new recruits of *C. intestinalis* were removed on a weekly basis, pulling panels out of the water for a short time period to do so; (2) Manipulated control, whereby panels were removed from the water each week (as in the experimental removal) but without *C. intestinalis* removal; and (3) Unmanipulated control, which remained in the water throughout the experiment. After 4 months, all of the panels were collected and analyzed to estimate species richness and relative abundance (percent cover) of sessile invertebrates and of *C. intestinalis*. Across all panels, species richness was negatively correlated with *C. intestinalis* abundance. The removal of *C. intestinalis* produced communities with significantly higher species richness than the controls. The overall species composition of treated and control panels was also distinctly different, with many species occurring more often in the absence of *C. intestinalis*, while others occurred more often on *C. intestinalis*-dominated panels. These data suggest that *C. intestinalis* both depress local species diversity and alter community assembly processes to fundamentally change sessile community composition.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Ascidian; *Ciona*; Fouling community; Impact; Invasion; Nonindigenous; Species richness

* Corresponding author. Tel.: +1 410 802 7925.

E-mail address: jcbalum@ucdavis.edu (J.C. Blum).

¹ Present address: Section of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA 95616.

² Present address: University of North Carolina Wilmington, 601 South College Road, Wilmington, NC 28403.

³ Present address: School for Marine Science and Technology, University of Massachusetts Dartmouth, 706 South Rodney French Blvd, New Bedford, MA 02744.

⁴ Present address: University of Delaware, Graduate College of Marine Studies, 233 Cannon Marine Studies Lab, Lewes, DE 19958.

⁵ Present address: Department of Environmental Science and Policy, University of California, One Shields Avenue, Davis, CA 95616.

1. Introduction

Sessile species, such as terrestrial plants or marine invertebrates, can exhibit dominant growth relative to other local species. For terrestrial plants, this often involves forming high-density or monospecific stands, frequently by overgrowing and shading surrounding competitors. While both native and introduced plant species can exhibit dominance, it is an important factor in an introduced plant's ability to become invasive. Monospecific stands of both native and introduced invasive plants tend to have negative consequences for local plant diversity (Ervin and Wetzel, 2002; Silliman and Bertness, 2004). Similar patterns have been observed among invasive marine algae, most notably *Caulerpa taxifolia* (Verlaque and Fritayre, 1994). Trends in the consequences of these stands for local animal diversity are less clear, with both increases and decreases recorded in the literature (e.g., Burger et al., 2003).

For sessile marine invertebrates, the mussel *Mytilus californianus* is the most familiar example of the effect of dominant growth; Paine (1966) demonstrated its negative effects on primary species diversity (i.e. species directly attached to the substrate) in the absence of its sea star predator. While in this case *M. californianus* was a native member of the ecosystem, similar dominant growth patterns have been observed for the introduced mussel *Mytilus galloprovincialis* in South Africa (Van Erkom Schurink and Griffiths, 1990; Robinson et al., 2005). Among other groups of marine invertebrates, numerous ascidian species also exhibit dominant growth in their introduced ranges, such as *Styela clava* (Lambert and Lambert, 1998), *Didemnum* sp. A (USGS, 2006), *Ciona intestinalis* (Carver et al., 2003), and, to some extent, *Molgula manhattensis* (JCB, pers. obs.). While these species are able to overgrow surrounding fauna or, in the case of the solitary species, form dense monospecific stands (e.g., Lambert and Lambert, 1998 for *C. intestinalis*), little information exists on the consequences of such growth patterns for the diversity of the surrounding community.

We selected the solitary tunicate *C. intestinalis* to measure effects of a high-density ascidian invasion on community structure. *C. intestinalis* has been introduced from northern Europe into bays on both coasts of North America, in New Zealand, the Mediterranean, Australia, Korea, Hawaii, Chile, South Africa, and Hong Kong (Lambert and Lambert, 1998, 2003; Castilla et al., 2005; Robinson et al., 2005). It exhibits dominant growth patterns in both its native range (Koechlin, 1977; Havenhand and Svane, 1991) and much of its

introduced range, where it is often a nuisance fouling species in aquaculture (Kang et al., 1978; Lesser et al., 1992; Castilla et al., 2005). The striking nature of *C. intestinalis* recruitment and its worldwide success as an invader underscore the importance of understanding its ecological effects.

In the highly invaded San Francisco Bay, we performed a manipulative field experiment to measure the effects of *C. intestinalis* dominance on the diversity of the local community at several spatial scales. *C. intestinalis* (hereafter, *Ciona*) was first recorded in San Francisco Bay in 1932 (Rodholm, 1932) and a 2001 survey observed several areas with approximately 100% cover of primary space (Ruiz et al. unpublished). Due to the importance of free space as a resource in fouling communities (Osman, 1977; Sutherland, 1981), we hypothesized that *Ciona* would have a negative effect on local sessile faunal species richness. A pilot study with *M. manhattensis* in Chesapeake Bay suggested that such an effect might also result in depression of the positive species-area relationship previously observed for marine sessile communities (Schoener and Schoener, 1981), as elsewhere (Rosenzweig, 1995).

2. Methods

2.1. *Ciona* recruitment at experimental site

Recruitment of new *Ciona* individuals was measured at Richmond Marina Bay Yacht Harbor, in the northeastern part of San Francisco Bay, from February 2002 to March 2003 by deploying five 13.7 by 13.7 cm fouling panels at 1 m depth for 1 month periods in locations evenly distributed around the site. Panels were retrieved monthly for analysis and replaced with new, bare panels. All panels used in this study were gray, 0.25 cm thick, sanded PVC.

Panels were individually isolated and transported between the field site and the laboratory in plastic bags filled with seawater and stored in a cooler. At the lab, panels were fixed in 10% buffered formalin and preserved in 70% ethanol. *Ciona* recruits were identified and counted on each panel to determine seasonal patterns of *Ciona* recruitment during the year of the experiment.

2.2. *Ciona*-removal experiment

To determine the effect of *Ciona*'s presence on fouling community species richness, we compared fouling panels from which we had experimentally removed *Ciona* to panels with naturally dense *Ciona*

growth. We included four different sizes of fouling panels to examine the effect of dense *Ciona* recruitment on species-area relationships.

The four sizes used were 7 by 7 cm (“small”), 13.7 by 13.7 cm (“medium”), 21.7 by 21.7 cm (“large”), and 34.3 by 34.3 cm (“extra-large”). These panel sizes represent multiples of approximately one, four, nine, and twenty-five times the area of the smallest panel (7 by 7 cm), respectively. Each panel was individually mounted on a brick and suspended from a floating dock at 1 m depth with the collecting surface of the panel facing downwards. The panels remained in the water, allowing organisms to settle on them and creating a community that we then manipulated.

Panels were assigned to one of three treatments: *Ciona*-removal, unmanipulated control, or manipulated control. Treatments were applied weekly for the duration of the experiment. The *Ciona*-removal treatment consisted of removing a panel from the water, placing it on the dock submerged in a tub of seawater, removing all visible *Ciona* with forceps, and then returning the panel to the water. We recorded the number of *Ciona* removed from each panel. Because both *C. intestinalis* and the closely related Japanese species *C. savignyi* were present at this site, yet are cryptic at juvenile size (less than 1 cm length), we did not distinguish between them when performing the *Ciona*-removal treatment. However, observations of recruitment and identification of adult individuals showed the overwhelming majority of *Ciona* present to be *C. intestinalis*. Manipulated control panels were treated in the same manner as *Ciona*-removal panels except that no *Ciona* individuals were removed, thus controlling for any artifacts of the manipulation involved in the *Ciona*-removal treatment. Unmanipulated control panels remained in the water undisturbed for the duration of the experiment.

The experiment took place on the docks of Richmond Marina Bay Yacht Harbor. This site was selected based on the high levels of *Ciona* recruitment observed in surveys conducted during the summer of 2001 (Ruiz et al. unpublished). Ten replicate panels in each size-treatment class (10 replicates * 3 treatments * 4 sizes = 120 panels) were deployed between June 24, 2002 and June 26, 2002. Panel locations and panel size-treatment combinations were distributed throughout the site using a stratified random scheme to ensure even spatial distribution. However, based on previous surveys (Ruiz et al. unpublished), we determined that the fouling community on the large, contiguous section of docks where we performed the experiment was fairly homogeneous in terms of species abundance and diversity. We

retrieved the panels after 17 weeks, between October 23, 2002 and October 26, 2002.

During the course of the experiment, 16 of the original 120 panels were lost. Most losses resulted from the weight of settled *Ciona* exceeding the tensile strength of the plastic cable ties used to attach the panels to their bricks. Therefore, losses were especially high among larger panels in the manipulated and unmanipulated control treatment classes. Due to the combination of differential panel loss and deployment error, the final set of 104 retrieved panels had treatment-size class replication ranging from 4 replicate panels per class (unmanipulated control-large) to 12 replicate panels per class (unmanipulated control-small). However, 8 out of 12 treatment-size classes contained between 7 and 10 replicate panels, with a median of 8.5 replicate panels across all classes.

Upon retrieval, panels were individually isolated and transported from the field site to a nearby laboratory in covered plastic bins filled with seawater. 79 panels were examined live within 24 h of retrieval and the remaining 25 panels were fixed in 10% buffered formalin, preserved in 70% ethanol, and examined over the course of the following 16 weeks.

To measure species richness, we recorded a comprehensive species list of sessile invertebrates for each panel, with voucher specimens of each species collected, fixed and preserved for identification by expert taxonomists. We used a two-way factorial ANOVA to test the effect of treatment and panel size on species

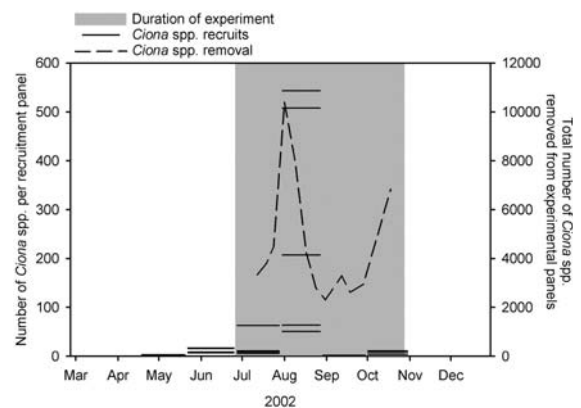


Fig. 1. *Ciona* recruitment to fouling panels. *Ciona* recruitment in Richmond Marina Bay Yacht Harbor, San Francisco Bay during 2002, measured two ways: (a) number of *Ciona* recruits on each of five 188 cm² PVC panels deployed for 1 month periods (left axis, solid bars; bar width represents the amount of time panels spent in the water); and (b) total number of *Ciona* removed weekly from 104 PVC panels ranging in size from 49 cm² to 1177 cm² as part of experimental treatment regime (right axis, line). The gray shaded area represents the duration of the experiment.

Table 1

Species found on experimental fouling panels set out in Richmond Marina Bay Yacht Harbor, San Francisco Bay, between June and October 2002

Bryozoa		Porifera	
<i>Bowerbankia aggregata</i>	C ^a	<i>Halichondria bowerbanki</i>	NIS
<i>Bowerbankia gracilis</i>	C	<i>Halichondria</i> sp.	C
<i>Bugula californica</i>	N ^b	<i>Haliclona</i> sp.	U
<i>Bugula neritina</i>	NIS ^c	<i>Mycale macginitiei</i>	N
<i>Bugula pacifica</i>	N	<i>Scypha</i> sp.	U
<i>Bugula stolonifera</i>	NIS	Polychaeta	
<i>Conopeum osburni</i>	C	Serpulidae	
<i>Conopeum seurati</i>	C	<i>Ficopomatus enigmaticus</i>	NIS
<i>Cryptosula pallasiana</i>	NIS	<i>Hydroides elegans</i>	NIS
<i>Electra monostachys</i>	C	<i>Hydroides gracilis</i>	C
<i>Schizoporella pseudoerrata</i>	NIS	Terebellidae	
<i>Scrupocellaria varians</i>	C	<i>Neoamphitrite</i> sp. A	C
<i>Tricellaria occidentalis</i>	C	Hydrozoa	
<i>Watersipora subtorquata</i>	NIS	<i>Obelia longissima</i>	C
Tunicata		Anthozoa	
<i>Aplidium</i> sp.	U ^d	<i>Diadumene</i> sp.	C
<i>Ascidia zara</i>	NIS	Bivalvia	
<i>Botrylloides diegensis</i>	N	<i>Mytilus</i> sp.	C
<i>Botrylloides violaceus</i>	NIS	Cirripedia	
<i>Botryllus schlosseri</i>	NIS	<i>Balanus improvisus</i>	NIS
<i>Ciona intestinalis</i>	NIS		
<i>Ciona savignyi</i>	NIS		
<i>Distaplia occidentalis</i>	N		
<i>Didemnum</i> sp. A	NIS		
<i>Diplosoma listerianum</i>	NIS		
<i>Molgula manhattensis</i>	NIS		
<i>Styela clava</i>	NIS		
<i>Styela plicata</i>	NIS		

^a C=species considered cryptogenic (*sensu* Carlton, 1996) in San Francisco Bay.

^b N=species considered native in San Francisco Bay.

^c NIS=species considered nonindigenous in San Francisco Bay.

^d U=unidentifiable samples; we were unable to identify these taxa precisely enough to determine their status.

richness, with multiple post-hoc comparisons performed using Fisher's Least Significant Difference (LSD). Analyses were conducted using the PROC GLM procedure of the SAS[®] software, version 9.1.2 (SAS Institute Inc., 2004).

As a measure of percent cover, 25-point counts were taken by placing a 5 by 5 grid over a randomly located

Table 2

Results of the analysis of variance (ANOVA) performed on the richness data

Source	df	MS	F	P
Treatment	2	176.9	30.63	<0.0001
Size	3	80.05	13.86	<0.0001
Treatment × size	6	6.606	1.14	0.3436
Error	92	5.776		

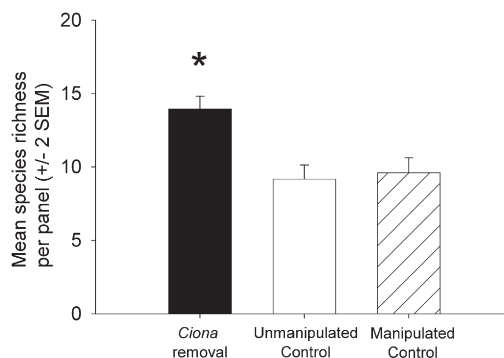


Fig. 2. Effect of *Ciona* removal on species richness. Mean sessile invertebrate species richness of PVC fouling panels assigned to one of three treatments: *Ciona* individuals removed from panel weekly (*Ciona* removal, filled bar, $n=36$), panel left undisturbed (Unmanipulated Control, empty bar, $n=35$), and panel handled in the same fashion as for *Ciona* removal, but with no *Ciona* removed (Manipulated Control, hashed bar, $n=33$). Asterisk indicates a significant difference using Fisher's LSD ($P<0.05$).

portion of each panel and recording the species present at each point. The total area covered by the grid was the same as the area of the smallest panel size (49 cm²). In order to control for edge effects and the increased amount of non-edge-adjacent space (36%) on extra-large panels, point counts were taken on two randomly-chosen portions of each extra-large panel, one located adjacent to the panel edge and the other located within the center portion of the panel. A single point count was then randomly selected from each pair for analysis such that half of the extra-large panels used point counts from the inner region and half used point counts from the outer region.

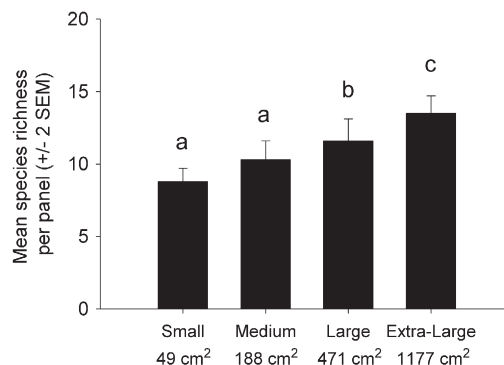


Fig. 3. Effect of panel size on species richness. Mean sessile invertebrate species richness of square PVC fouling panels of four sizes: 49 cm² (Small, $n=28$), 188 cm² (Medium, $n=29$), 471 cm² (Large, $n=21$), and 1177 cm² (Extra-Large, $n=26$). Dissimilar letters indicate a significant difference using Fisher's LSD ($P<0.05$).

Table 3

Incidence of species on fouling panels deployed in Richmond Marina Bay Yacht Harbor, San Francisco Bay, expressed as the percentage of panels from which the species was collected

Species	Percent incidence on experimental panels ^a		
	<i>Ciona</i> removal ^b	Unmanipulated control ^c	Manipulated control ^d
Tunicata			
<i>Didemnum</i> sp. A	92	↓↓↓	↓↓↓
<i>Styela clava</i>	72	↓↓↓	↓↓↓
<i>Ascidia zara</i>	64	↓↓	↓↓
<i>Diplosoma listerianum</i>	89	↓	↓
<i>Molgula manhattensis</i>	92	↓	↓
<i>Ciona savignyi</i>	6	↓	–
<i>Aplidium</i> sp.	3	–	–
<i>Styela plicata</i>	3	–	–
<i>Botrylloides diegensis</i>	3	No change	–
<i>Distaplia occidentalis</i>	–	↑	–
<i>Botryllus schlosseri</i>	86	↑	↓
<i>Botrylloides violaceus</i>	61	↑↑	↑↑
<i>Ciona intestinalis</i>	56	↑↑	↑↑
Bryozoa			
<i>Bugula stolonifera</i>	86	↓↓↓	↓↓↓
<i>Cryptosula pallasiana</i>	81	↓↓↓	↓↓↓
<i>Watersipora subtorquata</i>	58	↓↓	↓
<i>Conopeum seurati</i>	19	↓	↓
<i>Conopeum osburni</i>	17	↓	↓
<i>Bugula neritina</i>	100	No change	No change
<i>Bugula californica</i>	8	–	↓
<i>Bowerbankia gracilis</i>	17	–	–
<i>Bowerbankia aggregata</i>	3	–	–
<i>Bugula pacifica</i>	6	–	↑
<i>Tricellaria occidentalis</i>	3	–	↑
<i>Schizoporella pseudoerrata</i>	–	–	↑
<i>Electra monostachys</i>	–	↑	–
<i>Scrupocellaria varians</i>	–	↑	–
Porifera			
<i>Halichondria bowerbanki</i>	72	↓↓↓	↓↓
<i>Scypha</i> sp.	39	↓↓	↓↓
<i>Haliclona</i> sp.	17	–	–
<i>Mycale macginitiei</i>	–	–	↑
<i>Halichondria</i> sp.	22	↑	↑
Polychaeta			
<i>Hydroides elegans</i>	14	↓	↓
<i>Ficopomatus enigmaticus</i>	11	↓	–
<i>Hydroides gracilis</i>	3	–	–
<i>Neomamphitrite</i> sp. A	31	↑↑↑	↑↑↑
Hydrozoa			
<i>Obelia longissima</i>	3	–	–
Anthozoa			
<i>Diadumene</i> sp.	6	–	↓
Cirripedia			
<i>Balanus improvisus</i>	14	↓	↓
Bivalvia			
<i>Mytilus</i> sp.	11	–	↓

We compared community composition among the different treatments and panel sizes using the point count data in conjunction with multivariate routines (CLUSTER, MDS, ANOSIM, SIMPER) from the PRIMER-6 software package (Clarke and Gorley, 2006). A matrix of Bray–Curtis dissimilarities was produced for all panels using square root transformed, non-standardized point count data from which *Ciona* and botryllid tunicate cover was excluded. Square root transformation allows intermediately abundant species to contribute to the analysis in addition to the most abundant species, which would otherwise dominate the similarity measure (Clarke and Warwick, 2001). *Ciona* were excluded to avoid simply reporting that communities differed among the treatments because *Ciona* was absent from *Ciona*-removal panels and present on control panels. Meanwhile, we excluded botryllid tunicates due to concern that our sampling methods systematically underestimated their abundance on control panels. Examination of photos taken during panel retrieval confirmed that on control panels, botryllid tunicates were generally found growing on top of *Ciona*. This made the botryllids difficult to capture accurately using point counts, since the flexible *Ciona* tunics did not necessarily occupy one specific, fixed location. In contrast, on *Ciona*-removal panels, botryllids tended to attach to the panel itself as primary cover and were probably sampled much more accurately. With *Ciona* and botryllids excluded, the data matrices for four panels (two manipulated control panels, one size medium and one size extra-large, and two unmanipulated control panels, both size medium) became exceedingly sparse. The Bray–Curtis coefficient behaves erratically under these circumstances (Clarke et al., 2006), so we excluded these four panels from the multivariate analyses to prevent distortion of the results.

We classified our dissimilarity matrix with hierarchical agglomerative clustering using the unweighted pair group method with arithmetic means (UPGMA). Ordination of the matrix was accomplished by non-metric multidimensional scaling (nMDS) while ANOSIM (analysis of similarities) tested for differences

Notes to table

^a Control panel incidences are expressed relative to incidence on *Ciona* removal treatment panels (↑=+1–25%, ↑↑=+26–50%, ↑↑↑=+51–75%; ↓=–0–25%, ↓↓=–26–50%, ↓↓↓=–51–75%). Dashes (–) indicate 0% incidence.

^b *Ciona* spp. individuals removed from panel weekly ($n=36$).

^c Panel left undisturbed ($n=35$).

^d Panel handled in the same fashion as for *Ciona* removal, but with no *Ciona* sp. removed ($n=33$).

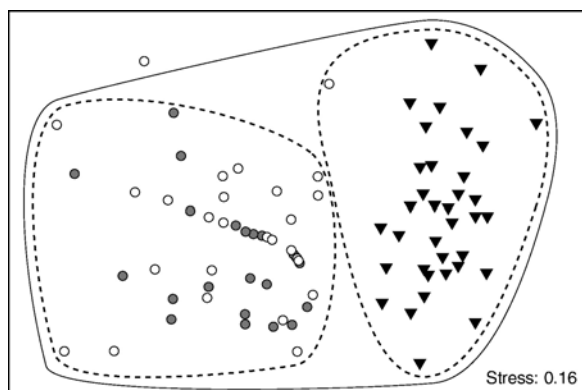


Fig. 4. Non-metric multi-dimensional scaling (nMDS) plot of community composition on panels based on square-root transformed abundance data with *Ciona* and botryllid tunicates excluded from the data matrix. Symbols indicate treatment: *Ciona* removal (triangles), Unmanipulated Control (open circles), and Manipulated Control (filled circles). Dashed lines indicate panels that are 40% similar according to CLUSTER analysis, while the solid line encloses panels that are 20% similar. Stress coefficient=0.16.

among treatment and panel size groups. ANOSIM is a permutation test that can be considered as a nonparametric analog to multivariate analysis of variance (MANOVA). It produces the R statistic, an absolute measure of the amount of separation between groups; R generally ranges between 0, indicating complete overlap and 1, indicating complete segregation (Clarke and Gorley, 2006). We used two-way crossed ANOSIM to examine treatment and size differences independently of each other and, where these differences were significant at the 0.05 level, to make pairwise comparisons between the various levels of each factor. The pairwise α was adjusted for multiple comparisons using the Bonferroni correction. When ANOSIM detected a significant grouping, a two-way crossed SIMPER (a similarity percentage procedure) was used to determine which species were most responsible.

3. Results

3.1. *Ciona* recruitment

Counts of *Ciona* individuals from recruitment panels showed a strong seasonal pattern, with a single peak in August (Fig. 1). The weekly counts of *Ciona* removed during the application of the *Ciona*-removal treatment accorded with this general pattern, but also showed a second, smaller peak during October (Fig. 1). This may represent recruitment from adult *Ciona* growing on the bricks and ropes associated with each panel. There

appears to have been no *Ciona* recruitment during the winter months.

3.2. *Ciona*-removal experiment

We found a total of 40 sessile animal species across all panels, of which 32 were either nonindigenous or cryptogenic (of uncertain native versus non-native status, *sensu* Carlton, 1996; Table 1). There were 3 taxa that could not be identified well enough to determine their biogeographic status. The predominant major taxa were Bryozoa (35% of all species found) and Tunicata (33% of all species found).

There were significant differences in species richness due to both treatment ($F_{2,92}=30.63$, $P<0.0001$) and size ($F_{3,92}=13.86$, $P<0.0001$) of panels, but the interaction between these factors was non-significant (Table 2). Panels from which *Ciona* was removed had significantly greater species richness than controls (Fig. 2). A positive species-area relationship was evident across all three treatments, with small and medium panels significantly less rich in species than large and extra-large panels (Fig. 3). Furthermore, many species were found more often on *Ciona*-removal panels than on panels in either control treatment. There were 8 species across 3 major taxa (Tunicata, Porifera, and Bryozoa) whose incidence on *Ciona*-removal panels was more than 25% greater than on either manipulated or unmanipulated control panels (Table 3). However, there were also two species found more often on panels in the control treatments: *Botrylloides violaceus* (>25%

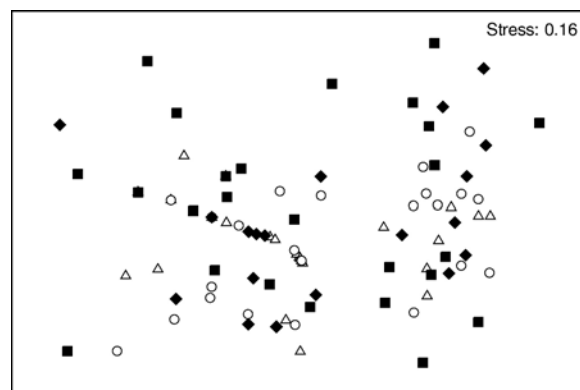


Fig. 5. Non-metric multi-dimensional scaling (nMDS) plot of community composition on panels based on square-root transformed abundance data with *Ciona* and botryllid tunicates excluded from the data matrix. Symbols indicate panel size: 7 by 7 cm ("small", open triangles); 13.7 by 13.7 cm ("medium", open circles), 21.7 by 21.7 cm ("large", filled diamonds), and 34.3 by 34.3 cm ("extra-large", filled squares). Stress coefficient=0.16.

Table 4

Results of the two-way crossed analysis of similarities (ANOSIM) performed on abundance data (square-root transformed, excluding *Ciona* and botryllid tunicates), with significant *P*-values in bold (Bonferroni corrected $\alpha=0.0083$)

Factor		<i>R</i>	<i>P</i>
Treatment	Global ANOSIM	0.562	0.001
	CR ^a :UC ^b	0.776	0.001
	CR:MC ^c	0.872	0.001
	UC:MC	0.011	0.378
Size	Global ANOSIM	0.088	0.009
	Small:Medium	0.035	0.217
	Small:Large	0.218	0.013
	Small:Extra-large	0.107	0.038
	Medium:Large	0.038	0.250
	Medium:Extra-large	0.016	0.349
	Large:Extra-Large	0.153	0.016

^a CR = *Ciona* removal treatment.

^b UC = Unmanipulated control treatment.

^c MC = Manipulated control treatment.

more often on controls) and *Neoamphitrite* sp. A (>50% more often on controls).

Community composition, as measured by point counts, differed dramatically between *Ciona*-removal and control panels, but only weakly between differently sized panels. Superimposing the groups identified via classification onto our nMDS results showed that *Ciona*-removal panels formed a cluster that split from

almost all manipulated and unmanipulated control panels at the 40% similarity level (Fig. 4). Meanwhile, there was a great deal of overlap among panel sizes (Fig. 5), and visual comparison of Figs. 4 and 5 shows that size-treatment combinations do not form distinct groups. The size classes did differ slightly in spread, with small and medium panels clustering more closely together than larger panels (Fig. 5).

Two-way crossed ANOSIM (treatment and panel size as factors) confirmed the ordination results. Treatments differed significantly in composition; specifically, *Ciona*-removal panels were significantly different from both the manipulated and unmanipulated controls (Table 4), with pairwise *R*-values greater than 0.75 indicating good separation (Clarke and Gorley, 2001). Meanwhile, the two control treatments were indistinguishable (Table 4). Panel size compositional differences were also significant, but the global *R*-value was quite low, and there were no significant pairwise size differences in composition (Table 4).

According to two-way crossed SIMPER, the most important and consistent distinguishing factors between the *Ciona*-removal treatment and the control treatments were the percent cover of bare space, the arborescent bryozoan *Bugula neritina*, the colonial tunicates *Diplosoma listerianum* and *Didemnum* sp. A, and the solitary tunicate *Ascidia zara*, all of which were

Table 5

SIMPER (similarity percentage) results showing which species made the greatest contribution to the dissimilarity between treatments

	Average abundance		Average dissimilarity	Average dissimilarity/SD ^a	Contribution (%)
	CR ^b	UC ^c	65.55		
Bare	0.10	<0.01	11.55	1.66	18.18
<i>Bugula neritina</i>	0.50	0.27	10.20	1.45	16.05
<i>Diplosoma listerianum</i>	0.05	0	7.31	1.22	11.50
<i>Didemnum</i> sp. A	0.03	<0.01	5.98	1.10	9.40
<i>Ascidia zara</i>	0.03	<0.01	5.20	0.91	8.18
Porifera	0.01	<0.01	3.86	0.89	6.08
<i>Styela</i> sp.	0.01	<0.01	3.74	0.79	5.88
<i>Bugula</i> spp.	<0.01	<0.01	3.59	0.79	5.64
					Total 80.91
	CR	MC ^d	65.66		
Bare	0.10	0	11.56	1.57	18.23
<i>B. neritina</i>	0.50	0.29	8.84	1.23	13.95
<i>D. listerianum</i>	0.05	<0.01	7.74	1.25	12.21
<i>Didemnum</i> sp. A	0.03	0	6.47	1.10	10.21
<i>A. zara</i>	0.03	0	6.16	1.00	9.71
<i>Styela</i> sp.	0.01	0	3.78	0.79	5.97
Porifera	0.01	0	3.65	0.85	5.76
<i>Neoamphitrite</i> sp. A	<0.01	0.01	3.60	0.71	5.68
					Total 81.71

^a SD = standard deviation.

^b CR = *Ciona* removal treatment.

^c UC = Unmanipulated control treatment.

^d MC = Manipulated control treatment.

greater on *Ciona*-removal panels (Table 5). Together, these five cover types accounted for nearly two-thirds of the difference between *Ciona*-removal panels and control panels (Table 5).

4. Discussion

Our results show that dense aggregations of *Ciona* depress overall species richness on a local scale and also change overall community composition, affecting both which species are present and how common they are. Whether measured by relative rate of occurrence or percent cover, certain species such as *Bugula stolonifera*, *Cryptosula pallasiana*, *Halichondria bowerbanki*, *Didemnum* sp. A, and *Styela clava* were associated with *Ciona*-free communities while other species such as *Botrylloides violaceus* and *Neoamphitrite* sp. A were associated with *Ciona*-dominated communities (Tables 3 and 5). Increased abundance of the universally occurring *B. neritina* in *Ciona*-free communities was also an important factor distinguishing these communities from *Ciona*-dominated ones (Table 5). Hence, the effect of *Ciona* dominance was to create a community where key species such as *B. neritina* were less abundant, many species were missing or rare, and a small number of *Ciona* associates became either more common, more abundant, or both (Tables 3 and 5). Therefore, although *Ciona* affected most sessile organisms negatively, there is evidence for its positive interaction with a subset of species. This implies that *Ciona*'s role in the community is more nuanced than that of a simple dominant.

Likely mechanisms driving the observed decline in species richness in *Ciona*-dominated communities are (a) high recruitment and successful space occupation by *Ciona* paired with (b) reduced recruitment onto or among *Ciona* individuals by other species. This two-pronged strategy appears to be an effective way of obtaining and retaining control over space. Many terrestrial and marine plants monopolize resources in a similar manner, rejecting competitors by both structural (overgrowth and exclusion of competitors from primary space, e.g., Boylen et al., 1999) and chemical means (allelopathy, e.g., Hierro and Callaway, 2003). *Ciona* has considerable particle clearance rates that may allow it to usurp food from competitors (Lesser et al., 1992; Petersen and Riisgard, 1992), but we did not examine this aspect of *Ciona* competitive ability, focusing more on structural factors. We found that initial *Ciona* recruitment onto bare panels was quite dense (Fig. 1) and was likely later supplemented by local recruitment due to this species' propensity for epibenthic settlement

and retention of fertilized eggs (Petersen and Svane, 1995). Once settled, *Ciona* seem to provide a poor substrate for other settlers, augmenting the tunicates' ability to swamp out other recruitment. Various ascidian species have significant antifouling chemical defenses (e.g., Davis, 1991; Hirose et al., 2001), and *Ciona* produces strong anti-microbial compounds that may restrict epibiosis (Findlay and Smith, 1995). However, physical defenses such as mucus and surface cell sloughing may ultimately be just as important (Davis, 1998). By and large, the tunics of the *Ciona* individuals we observed were unfouled, regardless of age. Indeed, on manipulated and unmanipulated control panels, the most successful other taxa were those that grew interstitially among the *Ciona*, such as *B. neritina* and terebellid worms, as well as botryllid colonial tunicates, which were able to grow on *Ciona* tunics.

Initially, we expected that severe species richness limitation by *Ciona* overgrowth might also be reflected in a flattened species-area response for *Ciona*-dominated communities, meaning that species richness would not increase with panel size. However, comparison of species richness across different panel sizes in *Ciona*-dominated (manipulated and unmanipulated control panels) and *Ciona*-free communities (*Ciona*-removal panels) revealed no significant interaction between *Ciona* dominance and species-area relationships (Table 2). Instead, larger panels continued to support more species, regardless of the degree of *Ciona* growth. Meanwhile, the results of our multivariate analyses indicate that there was no substantial change in community composition with size on a per-unit-area basis. Although the global *R*-value produced by 2-way ANOSIM for the four size groups was significant, it was also very low and there were no significant pairwise comparisons among sizes. We suggest that the differences in spread between treatment groups may be responsible for the significant global *R*-value, but that the value is so low that actual biological effects of size on composition are likely negligible. The lack of substantial compositional change with size supports the idea that differences in richness among sizes resulted from the addition of rare species, while the densities and identities of common species within each treatment remained the same as size increased.

In other systems, some seemingly dominant space occupiers, such as mussels, have been shown to increase diversity via their role as foundation species (Dayton, 1972, 1975). While mussels are able to crowd out other species at the primary substrate level, their shell surfaces and reef interstices provide new substrate for the settlement of other organisms and a relatively static,

stable habitat for mobile species (Witman, 1985, 1987; Thiel and Ullrich, 2002). In contrast, the apparently fouling-resistant *Ciona* seems to present a rather poor substrate for other sessile organisms. Furthermore, as compared to the decades-long lifespan estimated for *M. californianus* (Seed and Suchanek, 1992), *Ciona* is relatively short-lived. The maximum reported lifespan of individuals is 2 years, but a more typical lifespan is 1 year (Jackson, 2000); lifespan at our study site was 5 months at most (ALC, pers. obs.). The effect of *Ciona* cover on mobile fauna remains unexplored, although the arrangement and diversity of structural types in a habitat can influence habitat usage by mobile benthic fauna (e.g., Beck, 2000). The low diversity of structural types presented by a *Ciona* monolayer, as compared to a *Ciona*-free community, might lower the diversity of mobile fouling community associates. However, some mobile organisms might derive a benefit from *Ciona* cover if they were able to use the large, fleshy *Ciona* bodies as a refuge from predation. Although it is more logistically challenging to study mobile fouling community associates, we believe that this sort of work is critical to a more integrated understanding of *Ciona* effects and should be pursued.

It would also be valuable to extend future examinations of this community to longer time scales than we were able to study. While our results clearly show a striking within-season effect of *Ciona* dominance, the longer-term effects are not as obvious. At our study site, near-surface *Ciona* can disappear with the onset of winter rains and the corresponding drop in salinity. Similar seasonal dynamics have been widely reported, with the postulation that adults may persist at greater depths, recolonizing the shallower sub-tidal substrate in the spring (Lambert and Lambert, 1998, 2003). However, the dense growth pattern we described is not necessarily repeated at a given site from year to year. In fact, *Ciona* density seemed to be higher at the same site in 2001 and lower in 2003 (ALC, pers. obs.). In other sites in its invaded range, including Australia and New England, initially dense *Ciona* populations have declined as years passed (Kott, 1997; Hewitt et al., 2002; McDonald, 2004).

Although it remains to be seen how the diversity effects we documented will translate to greater spatial scales and longer time spans, high-density non-native ascidians clearly have the potential to cause broad changes in community composition. Our study examines one species at a small scale, but there are several other species of non-native ascidians reported to achieve similarly high levels of space occupation in their invaded ranges (Lambert and Lambert, 1998, 2003).

We hypothesize that these species may also have strong effects on species richness and community structure. With the growing number of tunicate invasions worldwide (Lambert, 2002), dramatic shifts in community richness could occur, with as yet unknown consequences for ecosystem function. The challenge remains to better understand these phenomena in order to better predict and respond to negative effects of species introductions.

Acknowledgements

Taxonomic expertise was graciously provided by Gretchen and Charlie Lambert (ascidians), Leslie Harris (terebellid worms) and Rolando Bastida-Zavala (serpulid worms). Christopher Brown (sponges), Natasha Hitchcock (bryozoans), and Esther Collinetti, Scott Cowan, and Kristen Larson (ascidians) also helped with voucher identifications. Similarly invaluable were Chaundra Schofield, Laurel Hillman, Scott Hackman, and Betsy Wells' tireless aid with fieldwork and Autumn Miller's enthusiastic counting of dead *Ciona*. Paul Fofonoff provided information on the invasion history of San Francisco Bay, Tami Huber helped with data management, and Mark Minton and Jeff Crooks gave thoughtful statistical advice. Comments from two anonymous reviewers greatly improved the manuscript. This study was funded by a grant from the National Sea Grant College Program. [SS]

References

- Beck, M.W., 2000. Separating the elements of habitat structure: independent effects of habitat complexity and structural components on rocky intertidal gastropods. *J. Exp. Mar. Biol. Ecol.* 249, 29–49.
- Boylen, C.W., Eichler, L.W., Madsen, J.D., 1999. Loss of native aquatic plant species in a community dominated by Eurasian Watermilfoil. *Hydrobiologia* 415, 207–211.
- Burger, J.C., Redak, R.A., Allen, E.B., Rotenberry, J.T., Allen, M.F., 2003. Restoring arthropod communities in coastal sage scrub. *Conserv. Biol.* 17, 460–467.
- Carlton, J.T., 1996. Biological invasions and cryptogenic species. *Ecology* 77, 1653–1655.
- Carver, C.E., Chisholm, A., Mallet, A.L., 2003. Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. *J. Shellfish Res.* 22, 621–631.
- Castilla, J.C., Uribe, M., Bahamonde, N., Clarke, M., Desqueyroux-Faúndez, R., Kong, I., Moyano, H., Rozbaczylo, N., Santelices, B., Valdovinos, C., Zavala, P., 2005. Down under the southeastern Pacific: marine non-indigenous species in Chile. *Biol. Invasions* 7, 213–232.
- Clarke, K.R., Gorley, R.N., 2001. PRIMER v.5: User Manual/Tutorial. PRIMER-E, Plymouth, U.K.
- Clarke, K.R., Gorley, R.N., 2006. PRIMER v.6: User Manual/Tutorial. PRIMER-E, Plymouth, U.K.

- Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd ed. PRIMER-E, Plymouth, UK.
- Clarke, K.R., Somerfield, P.J., Chapman, M.G., 2006. On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray–Curtis coefficient for denuded assemblages. *J. Exp. Mar. Biol. Ecol.* 330, 55–80.
- Davis, A.R., 1991. Alkaloids and ascidian chemical defense: evidence for the ecological role of natural products from *Eudistoma olivaceum*. *Mar. Biol.* 111, 375–379.
- Davis, A.R., 1998. Antifouling defence in a subtidal guild of temperate zone encrusting invertebrates. *Biofouling* 12, 305–320.
- Dayton, P.K., 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. In: Parker, B.C. (Ed.), Proceedings of the Colloquium on Conservation Problems in Antarctica. Allen Press, Lawrence, Kansas, pp. 81–95.
- Dayton, P.K., 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecol. Monogr.* 45, 137–159.
- Ervin, G.N., Wetzel, R.G., 2002. Influence of a dominant macrophyte, *Juncus effusus*, on wetland plant species richness, diversity, and community composition. *Oecologia* 130, 626–636.
- Findlay, C., Smith, V.J., 1995. Antimicrobial factors in solitary ascidians. *Fish Shellfish Immunol.* 5, 645–658.
- Havenhand, J.N., Svane, I., 1991. Roles of hydrodynamics and larval behavior in determining spatial aggregation in the tunicate *Ciona intestinalis*. *Mar. Ecol. Prog. Ser.* 68, 271–276.
- Hewitt, C.L., Martin, R.B., Sliwa, C., McEnulty, F.R., Murphy, N.E., Jones, T., Cooper, S. (Eds.), 2002. National Introduced Marine Pest Information System [Internet]. Available from: <http://crimp.marine.csiro.au/nimpis>. Date accessed: 5/27/2004.
- Hierro, J.L., Callaway, R.M., 2003. Allelopathy and exotic plant invasion. *Plant Soil* 256, 29–39.
- Hirose, E., Yamashiro, H., Mori, Y., 2001. Properties of tunic acid in the ascidian *Phallusia nigra* (Ascididae, Phlebobranchia). *Zool. Sci.* 18, 309–314.
- Jackson, A., 2000. *Ciona intestinalis*. A sea squirt. [Internet]. Marine Biological Association of the United Kingdom: Plymouth. Available from: <http://www.marlin.ac.uk/species/Cionaintestinalis.htm>. Date accessed: June 23, 2005.
- Kang, P.A., Bae, P.A., Pyen, C.K., 1978. Studies on the suspended culture of oyster, *Crassostrea gigas* in Korean coastal waters. 5. On the fouling organisms associated with culturing oysters at the oyster culture farms in Chungmu. *Bull. Fish. Res. Dev. Agency, Busan* 20, 121–127.
- Koechlin, N., 1977. Installation d'une épifaune a *Spirographis spallanzani* (Viviani), *Sycon ciliatum* (Fabricius) et *Ciona intestinalis* (L.) dans le port de plaisance de Lézardrieux (Côtes-du-Nord). *Cah. Biol. Mar.* 18, 325–337.
- Kott, P., 1997. The Tunicates. In: Shepherd, S.A., Thomas, I.M. (Eds.), Marine Invertebrates of Southern Australia, Part I. Government Printer, Adelaide, South Australia, pp. 1092–1255.
- Lambert, G., 2002. Nonindigenous ascidians in tropical waters. *Pac. Sci.* 56, 291–298.
- Lambert, C.C., Lambert, G., 1998. Non-indigenous ascidians in Southern California harbors and marinas. *Mar. Biol.* 130, 675–688.
- Lambert, C.C., Lambert, G., 2003. Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. *Mar. Ecol. Prog. Ser.* 259, 145–161.
- Lesser, M.P., Shumway, S.E., Cucci, T., Smith, J., 1992. Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. *J. Exp. Mar. Biol. Ecol.* 165, 91–102.
- McDonald, J., 2004. The invasive pest species *Ciona intestinalis* (Linnaeus, 1767) reported in a harbour in southern western Australia. *Mar. Pollut. Bull.* 49, 868–870.
- Osman, R.W., 1977. The establishment and development of a marine epifaunal community. *Ecol. Monogr.* 47, 37–63.
- Paine, R.T., 1966. Food web complexity and species diversity. *Am. Nat.* 100, 65–75.
- Petersen, J.K., Riisgard, H.U., 1992. Filtration capacity of the ascidian *Ciona intestinalis* and its grazing impact in a shallow fjord. *Mar. Ecol. Prog. Ser.* 88, 9–17.
- Petersen, J.K., Svane, I., 1995. Larval dispersal in the ascidian *Ciona intestinalis* (L.): evidence for a closed population. *J. Exp. Mar. Biol. Ecol.* 186, 89–102.
- Robinson, T.B., Griffiths, C.L., McQuaid, C.D., Rius, M., 2005. Marine alien species of South Africa — status and impacts. *S. Afr. J. Mar. Sci.* 27, 297–306.
- Rodholm, A.K., 1932. Contribution to the biology of the tube-building amphipod, *Corophium bonelli* (Milne-Edwards). University of California Berkeley, Master's thesis.
- Rosenzweig, M.L., 1995. Species Diversity in Space and Time. Cambridge University Press, Cambridge, UK.
- SAS Institute Inc., 2004. SAS OnlineDoc® 9.1.2. SAS Institute Inc., Cary, NC.
- Schoener, A., Schoener, T.W., 1981. The dynamics of the species area relation in marine fouling systems 1. Biological correlates of changes in the species area slope. *Am. Nat.* 118, 339–360.
- Seed, R., Suchanek, T.H., 1992. Population and community ecology of *Mytilus*. In: Gosling, E. (Ed.), The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture. Elsevier Science Publishers B. V., Amsterdam, pp. 87–169.
- Silliman, B.R., Bertness, M.D., 2004. Shoreline development drives invasion of *Phragmites australis* and the loss of plant diversity on New England salt marshes. *Conserv. Biol.* 18, 1424–1434.
- Sutherland, J.P., 1981. The fouling community at Beaufort, North Carolina: a study in stability. *Am. Nat.* 118, 499–519.
- Thiel, M., Ullrich, N., 2002. Hard rock versus soft bottom: the fauna associated with intertidal mussel beds on hard bottoms along the coast of Chile, and considerations on the functional role of mussel beds. *Helgol. Meeresunters.* 56, 21–30.
- USGS, 2006. Marine Nuisance Species, Genus Didemnum, colonial tunicate; ascidian; sea squirt [Internet]. USGS: Woods Hole, MA. Available from: <http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/>. Date accessed: April 15, 2006.
- Van Erkom Schurink, C., Griffiths, C.L., 1990. Marine mussels of Southern Africa: their distribution patterns, standing stocks exploitation and culture. *J. Shellfish Res.* 9, 75–86.
- Verlaque, M., Fritayre, P., 1994. Mediterranean algal communities are changing in the face of the invasive alga *Caulerpa taxifolia* (Vahl). *Oceanol. Acta* 17, 659–672.
- Witman, J.D., 1985. Refuges, biological disturbance, and rocky subtidal community structure in New England. *Ecol. Monogr.* 55, 421–445.
- Witman, J.D., 1987. Subtidal coexistence: storms, grazing, mutualism, and the zonation of kelps and mussels. *Ecol. Monogr.* 57, 167–187.