Recipient Environment More Important than Community Composition in Determining the Success of an Experimental Sponge Transplant

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Abstract

Human activities have inflicted profound damage upon many ecosystems, and ecologists are now seeking effective means of restoring ecosystems to their natural state. Industrial ports and harbors are highly modified and often depauperate in native fauna. They are typically characterized by poor water quality and modified community composition, both of which may hinder attempts to reintroduce native species. Here, we conducted a field experiment to separate the effects of the recipient environment and community composition on the success of endemic sponge explants in Port Kembla Harbor, NSW, Australia. A reciprocal transplant was conducted between communities originating from six sites that varied in water quality and community composition, enabling us to assess the relative factors simultaneously. A colony of the endemic sponge *Tedania anhelans* was then inserted into the center of each community, and we quantified the survival, growth, and metal bioaccumulation of sponges over three months. Endemic sponges consistently performed better against resident assemblages when water quality was good. Sponges transplanted to cleaner sites had over double the survivorship and approximately three times the surface area of sponges transplanted to disturbed sites. These patterns were independent of community composition. Bioaccumulation of metals in sponges was correlated with survival; however, other factors such as turbidity may be required to explain sponge mortality at some sites. This study adds to evidence that remediation of the physical and chemical environments may be a prerequisite for biological remediation and demonstrates the value of experimental transplants in assessing restoration potential.

Key words: **bioaccumulation, community composition, competition, native animals, overgrowth, recipient environment, reciprocal transplant, sponge, water quality.**

Introduction

Port and estuarine environments have been subject to drastic anthropogenic change for the benefit of industry and urbanization (Kennish 2002). Many ports now carry increased sediment and pollutant loads and suffer ongoing disturbance to their natural hydrological regimes. Despite widespread recognition of the potential problems that human activities may cause biotic assemblages in these areas, there has been relatively little quantification or mitigation of the impacts and even fewer attempts to restore degraded ecosystems. As a result, the baseline diversity and functioning of these assemblages are becoming impoverished (Jackson et al. 2001), and our ability to predict the success of restoration programs remains poor (Peterson & Lipcius 2003).

Restoration ecology has emerged as a growing discipline that aims to return ecosystems to their natural state (Young 2000). Restoration involves the remediation of physical (habitat structure and quality, disturbance regime) and/or biological (species identity and biodiversity) components of an ecosystem and must also consider potential interactions of these components. Much of the recent progress in restoration ecology has been spurred by legislative and regulatory requirements placed on developers to mitigate and restore natural systems (Peterson et al. 2003). Most of this work has been conducted in terrestrial or freshwater systems and some intertidal wetlands, reflecting the close association between the general public and these areas. Beyond the sight of most urban dwellers (and consequently their public representatives) are the subtidal marine and estuarine systems. Although human impacts on marine, particularly coastal, ecosystems are often as drastic and costly as those inflicted upon terrestrial ecosystems (Jackson et al. 2001; Kennish 2002), our conceptual framework for marine restoration is considerably less developed.

Many marine restoration studies have resulted from high-profile environmental incidents such as ship groundings and pollutant spills (Peterson et al. 2003) and focus on the practical techniques of reestablishing structural components of habitats such as corals (Rinkevich 1995; Bruckner & Bruckner 2001; Epstein et al. 2003), sea

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grasses (West et al. 1990; Bull et al. 2004; Fishman et al. 2004; Williams & Davis 2004), algal beds (Terawaki et al. 2003), and artificial reefs (Wilson et al. 2002; Piazza et al. 2005). Only recently have marine researchers begun to address some of the theoretical issues that dominate the terrestrial literature (Epstein et al. 2003), although several studies have provided valuable insights into factors affecting restorations in particular habitats. Zedler (2000) gives an excellent review of theoretical advances in wetland restoration, and significant progress has been made in understanding sea grass restorations by applying concepts from landscape ecology (Bell et al. 2001). Other studies have demonstrated the value of rigorous monitoring to observe community trajectories in constructed salt marshes and coral reefs (Craft et al. 2003; Lirman & Miller 2003), which has allowed them to identify critical processes in the development of artificial habitats. These contributions have started to build the necessary framework for successful restorations, but there remain key aspects of restoration ecology that have not been adequately explored in marine systems. One such aspect is the effects of existing community composition on biological restorations and interactions between community composition and environmental quality.

The majority of industrial and urban development occurs around bays and estuaries, and it is these biologically productive systems that are subject to increasing anthropogenic impacts (Kennish 2002). Filter-feeding invertebrates represent important assemblages in these areas, inhabiting hard substrates such as rocky reefs (Carballo & Naranjo 2002). These assemblages are biologically diverse, often comprising six or seven phyla in less than 100 cm^2 (Johnston & Keough 2005). They also play important functional roles in the system, providing food and habitat for fish and mobile invertebrates and contributing to improved water quality through filter feeding in areas with little oceanic flushing (Hily 1991).

Encrusting sponges are prominent members of temperate reef assemblages. Arguably, the simplest of animals, this diverse phylum feeds by filtering particles as small as ultraplankton out of the water (Pile et al. 1996). They are generally good competitors for the major limiting resource (space) and are a predictable, sometimes dominant, component of mature hard-substrate assemblages (Jackson 1977; Kay & Keough 1981; Russ 1982; Nandakumar et al. 1993; Fairfull & Harriott 1999). Their growth rates often depend on flow rates (Wilkinson & Vacelet 1979; Bell 2002) and water temperature, and they are particularly susceptible to poor water quality (Roberts et al. 1998). Delicate filter-feeding mechanisms may become clogged under heavy suspended sediment loads, and they have been shown to rapidly accumulate heavy metals in their tissues under polluted conditions (Hansen 1995; Cebrian et al. 2003).

Restoration ecology aims to simultaneously restore both diversity and ecosystem function to impacted systems, although in many cases, the two are difficult to achieve simultaneously. In degraded marine hard-substrate assemblages, the successful reintroduction of endemic sponges is likely to improve both biodiversity and ecosystem function. Where the reintroduction of organisms is the aim of restoration process, then success is likely to depend on both environmental and biological influences in the area (Bond & Lake 2003). Just as predators, competitors and disease are likely to affect the survival of experimental reintroductions, so too are environmental conditions such as water quality and flow rates (Fonseca & Bell 1998; Peterson & Lipcius 2003). In order to maximize the growth and survival of explants and predict the success of future reintroductions, the influence of biological and environmental conditions needs to be distinguished.

Our aim was to assess the relative importance of (1) the recipient environment and (2) community composition on the success of experimental reintroduction of an endemic sponge into a degraded port. We grew hard-substrate marine communities at various sites throughout an industrialized harbor. Water quality varied between sites, as did community composition. We then conducted a reciprocal transplant of communities between sites, such that the factors of community composition and recipient environment were fully crossed. An endemic sponge was then inserted into each community, and we quantified the survival, growth, and metal bioaccumulation of the sponge over 14 weeks. By comparing the effect of the initial site (where communities originated) to the final site (where communities were transplanted to) on sponge growth and survival, we could gauge whether community composition or environmental conditions were the primary determinants of establishment. If sponges survived and grew similarly within but not between sites, then environmental conditions are likely to be important to sponge establishment. If sponge survival and growth differed within a site (i.e., depending on the origin of the assemblage they had been placed into), then we would predict that the identity and/or composition of the surrounding community will play an important role in determining the success of any full-scale sponge restoration attempt.

Methods

Study Site

Port Kembla Harbor, NSW (lat 34°28′S, long 150°54′E), is an industrialized harbor with a history of heavy-metal pollution (He & Morrison 2001). It is subject to frequent shipping activities and its surrounds include a copper smelter, steel smelter, coal- and grain-exporting terminals, and a fertilizer-manufacturing plant. The port is divided into an Inner and Outer Harbor, connected via a narrow shipping channel (Fig. 1). Water quality in the Inner Harbor is poor, and heavy-metal concentrations in both the water and the sediment frequently exceed Australian guidelines (ANZECC & ARMCANZ 2000; He &

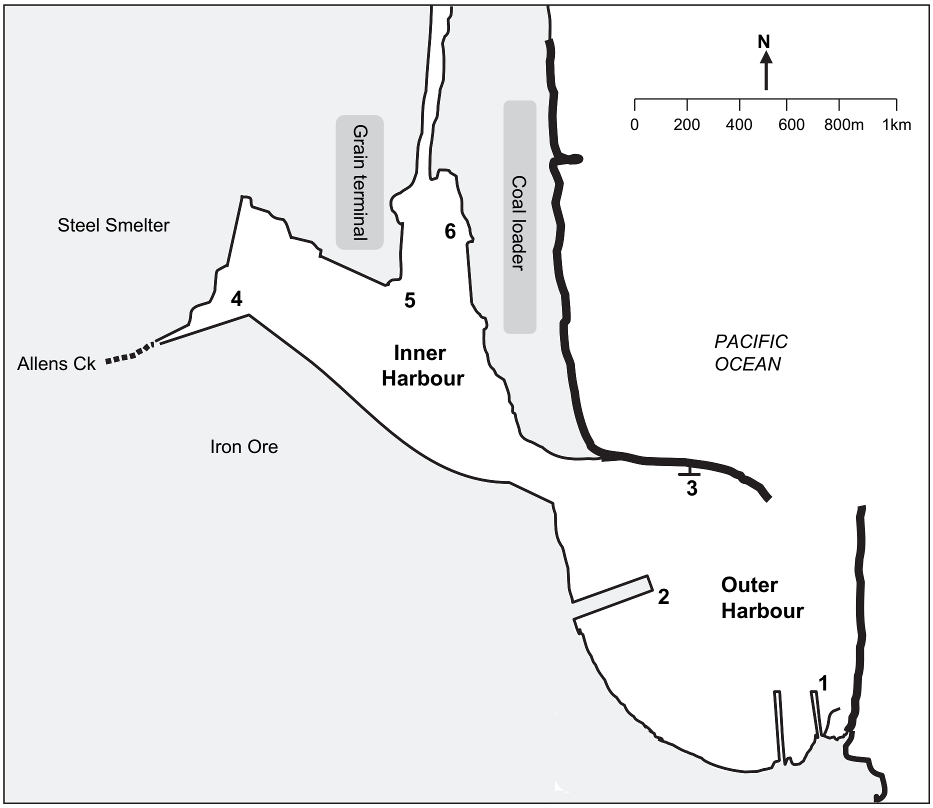

Figure 1. Map of Port Kembla Harbor showing sponge deployment sites.

Morrison 2001). The Outer Harbor is considered less polluted (He & Morrison 2001), with better flushing and fewer pollution point sources.

Experimental Design

Experimental Communities. On 3 March 2004, 21 settlement plates were deployed at each of the six sites in Port Kembla Harbor to obtain replicate hard-substrate communities. Three sites were in the Inner Harbor, and three in the Outer Harbor (Fig. 1). At each site, Perspex settlement plates (11 × 11 × 1 cm) were attached to the underside of two 60 × 60–cm polyvinyl chloride (PVC) backing panels (see Johnston & Keough 2002, for panel design) and suspended from wharf structures at a depth of 2 m below low-water mark. Plates were left undisturbed for a period of five months to develop natural assemblages that included barnacles, serpulids, bryozoans, and ascidians. Plates were facing down to avoid confounding differences in sedimentation rates between sites.

Preparation of Sponge Colonies. Laminar colonies (approximately 2 cm in height) of the Orange encrusting sponge (*Tedania anhelans*) were collected from the Container Wall of Botany Bay, NSW (lat 33°58′S, long 151°12′E), on 27 May 2004. *Tedania anhelans* has a widespread distribution throughout coastal NSW and is a typical component of local functioning assemblages (Roberts & Davis 1996; Knott et al. 2004). It occurs both on natural rocky reefs and on artificial substrates (Knott et al. 2004). A total of 21 sponges have been recorded from within Port Kembla Harbor including one *Tedania* species (Pollard & Pethebridge 2002), but species-level identification and abundance data are not currently available. Colonies of the sponge were cut into 140 replicate discs (4.7 cm diameter) using a circular cutter, and each sponge disc was secured to a plastic disc (5.1 cm diameter) with rubber bands (Fig. 1). This technique was modified from a study of the effect of orientation on the growth rates of *T. anhelans* by Knott et al. (2006). Each plastic disc had a stainless steel bolt attached through the center to allow it to be fastened to a backing plate. These units will hereafter be referred to as "sponges." Sponges were attached to PVC backing plates and were suspended upside down from Kurnell Pier, Botany Bay, NSW (lat 34°00′S, long 151°13′E), at a depth of 2 m below low-water mark. The collection, cutting, and redeployment of sponges took place over a six-hour period on one day so as to minimize disruption to the organisms. Sponges were kept in aerated local seawater during cutting, attachment, and transport. Kurnell Pier is well flushed with oceanic seawater, and heavy-metal concentrations are not elevated above background (Piola & Johnston 2005). Sponges were left undisturbed at this site for four weeks to allow recovery from the disturbance of collection and cutting and to encourage attachment to experimental discs.

Reciprocal Transplant and Sponge Deployment. On 27 and 28 July 2004, a reciprocal transplant was conducted with the settlement plate assemblages in Port Kembla Harbor. Replicate assemblages ($n = 3$) were randomly chosen from each of the six sites and transferred to every other site, and a further three were removed and returned to their original site as a procedural control for the disturbance of the transplant (total $n = 18$ transplanted from each site). So, for example, $n = 18$ assemblages were collected from site 1, and three of these assemblages were deployed at each of the sites 1–6 (Fig. 1). This was repeated for each site. A further three settlement plate assemblages were collected from each site at this time and returned to the laboratory to determine assemblage composition prior to the transplant. During the transplant process, a 5.1-cm diameter circle was cleared in the center of each assemblage and a sponge was inserted into each community (6 sites $\times$ 18 assemblages = 102 sponge explants; Fig. 2). Sponges had been collected from their recovery site at Kurnell Pier and transported to Port Kembla Harbor in aerated seawater. Assemblages with sponges were left undisturbed for three months before they were collected for census on 2 November 2004.

Water samples were taken from the surface at each of the six sites on three separate occasions throughout the experimental period (16 June, 20 July, and 2 November). Samples were collected in acid-washed, high-density polypropylene containers. Water samples were acidified with 70% HNO_3 and kept refrigerated prior to analyses. Water samples were analyzed for total concentrations of Zn, Pb, and Fe by the N.A.T.A.-accredited National Measurement Institute. Basic water quality parameters (pH, salinity, dissolved oxygen, turbidity, and temperature) were measured on the same dates using a YeoKal Model 603 meter.

Plaster blocks were deployed once at each site for a comparison of flow rates. Plaster blocks were made by mixing 15 g of dental plaster with 11 mL of water, pouring into a 4-cm diameter plastic mould and inserting the head of a stainless steel bolt into the plaster. Plaster blocks were dried at a constant heat of 40°C for four days and individual weights were taken. Replicate blocks ($n = 4$) were deployed on 30 May 2005 at all six sites within the harbor for a period of three days (72 hours). Blocks were attached to the underside of backing plates at the same depth and in the same manner as experimental assemblages. Blocks were retrieved after three days and oven dried at 40°C for four days. Weights were again recorded and the proportion of each block that had dissolved was calculated.

Upon final collection of the assemblages, digital photographs were taken of each plate with sponge intact. Sponges were then removed using acid-washed (10% HNO_3) plastic utensils and placed in separate ziplock bags. Sponges were transported to the laboratory in insulated containers with ice. At the laboratory, each sponge was rinsed twice briefly in MilliQ-filtered water to remove sediment, the sponge surface was blotted dry, and a wet weight was recorded. Samples were then placed in clean ziplock plastic bags and deep frozen at -70°C. Immediately prior to analysis, samples were placed in acid-washed plastic containers in a drying oven and dried at 40°C for 48 hours. Samples were then crushed into a fine powder using a metal-free graphite mechanical homogenizer. Samples from each site were combined to form composite samples. Subsamples of 0.4 g of sponge powder were weighed on a fine balance and used for analysis. Powdered samples were added to 5 mL of super concentrated distilled HNO_3, 2 mL of MilliQ water, and 2 mL of hydrogen peroxide in acid-washed Teflon digestion vessels. Samples were digested by microwave digestion at 190°C for 20 minutes. Samples were retrieved and made up to 10 mL using MilliQ-filtered water and analyzed with an inductively coupled atomic emission spectrometer. Standard Reference Material 121 (Australian Soil and Plant Analysis Council State Chemistry Laboratory, Victoria, Australia) was analyzed as a quality control along with sample blanks. Recovery was within the acceptable range (±10%). Results are expressed in μg/g of metal with respect to sponge tissue (dry weight).

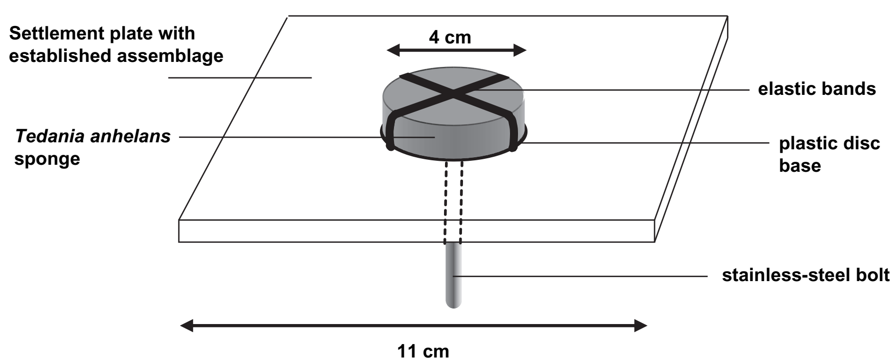

Figure 2. Diagram showing method of inserting replicate sponge colonies into preexisting communities.

Census and Analysis

The encrusting growth habit of this sponge allowed us to determine sponge growth by measuring surface area in the digital photographs. PixelLink* software was used to quantify the two-dimensional surface area occupied by living sponge tissue (differentiated from dead tissue by color). Some sponges at site 1 were wholly covered by colonial ascidians but on dissection of the community were later found to be alive, so these were considered missing replicates rather than zero values when surface area was analyzed.

Assemblage composition was quantified in two ways. Plates collected from each site prior to the reciprocal transplant were subsampled alive under a dissecting microscope. Twelve 1 × 1–cm quadrats were censused along two transects that dissected each plate; care was taken to avoid edge effects and areas that would later be covered by the sponge insertion. Counts were made of barnacles, serpulids, arborescent bryozoans, and solitary ascidians; whereas, percent cover was recorded for encrusting bryozoans, colonial ascidians, and bare space (no sponges were observed). Settlement plate assemblages collected after three months of experimental sponge deployment could not be censused in the same way due to a breakdown in the flowing seawater aquarium system that destroyed all assemblages within 24 hours of return to the laboratory. We relied on digital photographs taken at the time of collection to quantify assemblage composition on these plates. Fifty random points were superimposed over each digital photograph, and counts were made of the fauna occurring under each point to provide percent cover. Care was again taken to avoid edge effects and areas that had been covered by the sponge implant. Fauna was recorded in the categories of barnacles, colonial ascidians, serpulid polychaetes, encrusting bryozoans, solitary ascidians, and bare space (this was a nondescript matrix comprising silt, microorganisms, plate surface, and dead barnacles). Again, no sponge cover was recorded besides the experimental sponge.

Sponge survival was analyzed using a Kruskal–Wallis nonparametric test with a single fixed factor of Harbor (Inner or Outer). All other measured variables were continuous and were therefore analyzed using analysis of variance (ANOVA). Initial assemblage composition, metal concentrations in water samples, and plaster block dissolution were analyzed with the fixed factor of Harbor (Inner or Outer) and the random factor of site nested within harbor. Sponge growth and assemblage composition after the reciprocal transplant were analyzed with two fixed factors (Initial Harbor and Final Harbor) and two random, nested factors (site within Initial Harbor, site within Final Harbor). Where no site effects were found and the probability values for these terms were greater than 0.225, data were pooled and analyzed with a two-factor ANOVA using the factors Initial Harbor and Final Harbor. ANOVA were performed using SYSTAT 10 (SPSS, Inc., Chicago, IL, USA). Data were assessed for normality and homogeneity of variance using frequency histograms of residuals and plots of residuals versus means, respectively (Quinn & Keough 2002). Data were square root transformed where this improved homogeneity. Multivariate analyses were performed using permutational multivariate analysis of variance (Anderson 2001), and multidimensional scaling (MDS) ordination plots were created using Bray–Curtis similarities and the software Primer (Clarke & Warwick 1994). Multivariate analyses revealed no additional patterns, and only univariate data are therefore presented to avoid repetition.

Results

Sponge Growth and Survival

The survival of sponges transplanted into the Outer Harbor (75%) was almost double that transplanted to the Inner Harbor (40%; Fig. 3a, Kruskal–Wallis $p < 0.000$).

Clear patterns were found in the two-dimensional area of sponges, for which we could differentiate between

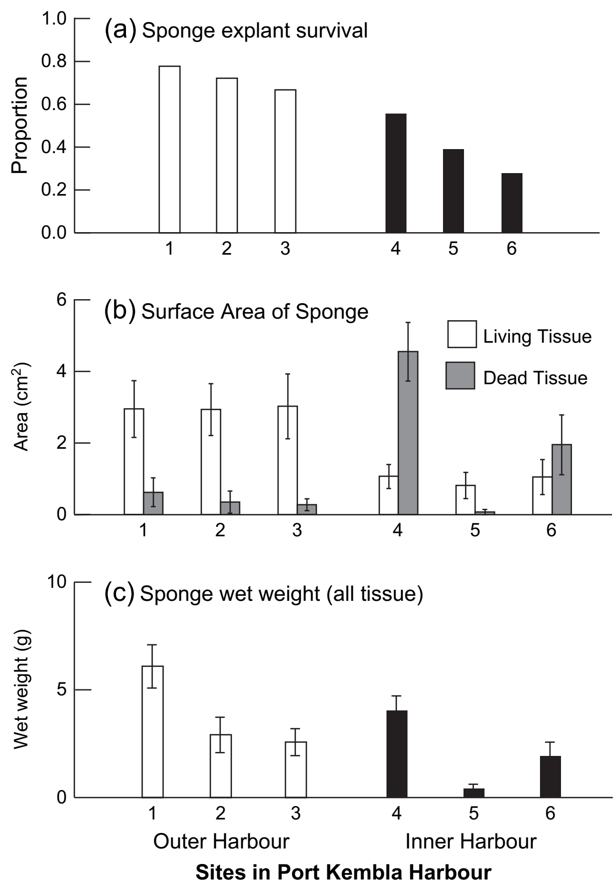

Figure 3. (a) Proportion of sponge explants surviving, (b) surface area of living and dead sponge, and (c) sponge wet weight (all tissue) after 14 weeks deployment at sites within the Inner and Outer Harbors of Port Kembla.

living and dead tissue. Living sponges in the Outer Harbor occupied more than three times the area of living sponges in the Inner Harbor, and there was no significant variation between sites within harbors (Table 1; Fig. 3b). We did not detect effects of the initial assemblage composition (Initial Harbor or initial site within Initial Harbor) on live sponge tissue, which suggests that community composition did not affect sponge growth or survival. The surface area of dead sponge tissue in the Inner Harbor was variable, as sponges at site 4 retained far more dead tissue than those at site 5 (Table 1; Fig. 3b). In general, sponges did not grow during the deployment period.

Sponge wet weight, which includes both living and dead tissue, was highly variable between sites (Table 1; Fig. 3c). Wet weight also includes sponges from site 1 that were wholly covered by colonial ascidians when photographed but on dissection of the assemblage were later found to be alive underneath the ascidians. This accounts for the greater wet weight of sponges at site 1 (Fig. 3c).

Bioaccumulation of Metals

The concentration of metals (Zn, Ni, Pd, and Fe) accumulated by sponges is shown in Figure 4. The variation between sites was similar for all metals. Although Outer Harbor sites showed consistently low concentrations, sites 5 and 6 in the Inner Harbor showed very high and low concentrations, respectively.

Water Quality and Flow Rates

There were elevated levels of iron in the waters of the Outer Harbor relative to the Inner Harbor (Table 2; Fig. 5). No differences were found for concentrations of lead or zinc. Flow rates differed between sites within the harbors. Sites 2 and 3 in the Outer Harbor experienced relatively higher flow rates than sites 1, 4, 5, and 6, which were similar (Table 2; Fig. 5d). Basic water quality parameters (pH, salinity, DO) were similar across sites and times (Table 3). Temperature and turbidity were slightly elevated at site 5 closest to Allan's creek and the steelworks' cooling water outlet (Table 3).

Assemblage Composition Prior to Transplant

Prior to the reciprocal transplant, there was a higher density of barnacles within assemblages in the Inner Harbor (Table 4; Fig. 6). There was a trend toward less available bare space in the Outer Harbor assemblages; however, this was not significant (Table 4; Fig. 6). The percent cover of encrusting bryozoans and the density of arborescent bryozoans and serpulid polychaetes differed between sites but not harbor (Table 4; Fig. 6). Solitary ascidians were rare and there was no significant difference in their densities between harbors or sites within harbors (Table 4; Fig. 6).

Final Assemblage Composition

Taxonomic groups showed significant variation between sites within harbors (Table 5). The final site position influenced the percent cover of colonial ascidians which dominated assemblages at sites 1 and 2 (Table 5; Fig. 7), often overgrowing sponges but not yet causing mortality. The final site also influenced available bare space which was lowest (approximately 10%) in assemblages transplanted to site 1 in the Outer Harbor and highest (approximately 50%) within those transplanted to site 6 in the Inner Harbor (Table 5; Fig. 7). Barnacle cover varied between final positions peaking at 10% in assemblages transplanted to Inner Harbor site 5 (Table 5; Fig. 7). Both the initial and the final sites affected the abundance of serpulids (Table 5; Fig. 7). Serpulid cover was highest (approximately 30%) in assemblages transplanted to Outer Harbor site 5 (Table 5; Fig. 7); however, their initial site also influenced abundances with the lowest cover (approximately 10%) in assemblages that originated from site 4 in the Inner Harbor (Table 5; Fig. 7). Solitary ascidians occupied eight or

Table 1. ANOVA of the living and dead surface areas and wet weights of transplanted sponges in Port Kembla Harbor.

	Area Alive			*Area Dead*			*Wet Weight*		
Source	*df*	*Mean Square*	*p*	*df*	*Mean Square*	*p*	*df*	*Mean Square*	*p*
IH	1	0.023	0.880	1	1.325	—	1	74.278	—
FH	1	19.596	**<0.001**	1	11.529	—	1	0.676	—
IH × FH	1	0.041	0.832	1	2.947	—	1	0.171	—
Initial site (IH)	—	—	—	4	0.566	0.338	4	59.371	0.770
Final site (FH)	—	—	—	4	6.681	**<0.001**	4	3.598	**<0.001**
IH × final site (FH)	—	—	—	4	0.539	0.363	4	4.283	0.708
FH × initial site (IH)	—	—	—	4	0.625	0.287	4	6.638	0.508
Initial site (IH) × final site (FH)	—	—	—	16	1.275	**0.003**	16	9.881	0.261
Error	97	0.920		65	0.489		68	7.957	

Data for surface areas are square root transformed; wet weight is untransformed. There were no significant site effects in the full ANOVA model for sponge surface area alive and $p > 0.225$ so the data from sites were pooled and reanalyzed for this dependent variable. There are no appropriate *F* tests for the main effects in the full ANOVA model due to the two random factors (Downes et al. 2002). Brackets indicate nesting. Significant differences at $\alpha = 0.05$ are highlighted in bold. IH, Initial Harbor; FH, Final Harbor.

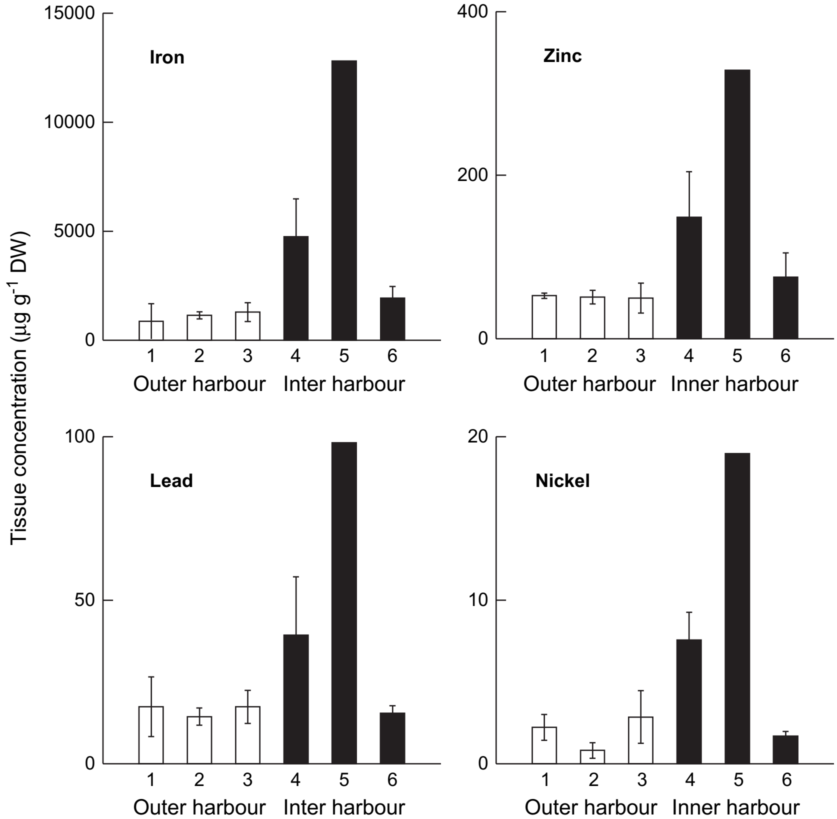

Figure 4. Mean total concentrations (μg/g dry weight ± 1 SE) of metals in *Tedania anhelans* sponge tissue at six sites in Port Kembla Harbor. Replication was not possible for site 5 due to lack of sponge material.

nine times the space in assemblages that originated from site 1 relative to any other site although this was only approximately 10% cover (Table 5; Fig 7). Encrusting bryozoans were almost absent from plates originating from sites 5 and 6 (Table 5; Fig 7). Only one sponge occurred in our experimental assemblages. A cryptogenic species from the order Leucosolenia (genus *Sycon*) was recorded from plates in both the Inner and the Outer Harbors. This sponge covered less than 1% of any settlement panel at any one site, and therefore, data for this species were not analyzed.

Table 2. ANOVA for metals in water and plaster block dissolution rates at all sites within Port Kembla Harbor.

	Source	*df*	*MS*	*p*
Iron	Harbor	1	80.113	**0.035**
	Site (harbor)	4	8.112	0.255
	Error	12	5.297	
Lead	Harbor	1	0.275	0.651
	Site (harbor)	4	1.156	0.057
	Error	12	0.372	
Zinc	Harbor	1	8.565	0.214
	Site (harbor)	4	3.927	0.672
	Error	12	6.583	
Plaster	Harbor	1	0.210	0.060
	Site (harbor)	4	0.031	**<0.001**
	Error	18	0.001	

Data have been square root transformed prior to analysis. Significant differences at $\alpha = 0.05$ are highlighted in bold.

Discussion

Species reintroductions will often be necessary because of regional constraints such as a lack of source population or limited dispersal ability of organisms (i.e., failure of the Field of Dreams hypothesis—Palmer et al. 1997). Experimental tests of species reintroductions are therefore necessary to establish the feasibility of restoration given ongoing disturbances (Bond & Lake 2003). Restoration studies in marine systems have examined the success of various explants; however, to our knowledge, this is the first experimental reintroduction of a marine sponge. Survivorship and growth of sponge explants depended mostly on position within Port Kembla Harbor. In general, sponges transplanted to sites within the more disturbed Inner Harbor had far lower survival and greater reductions in size than those transplanted to the Outer Harbor. This pattern was evident, regardless of the composition of the assemblage that the sponges were transplanted into. Our results suggest that water quality parameters and not community composition will be the major determinant of sponge explant success in this degraded port.

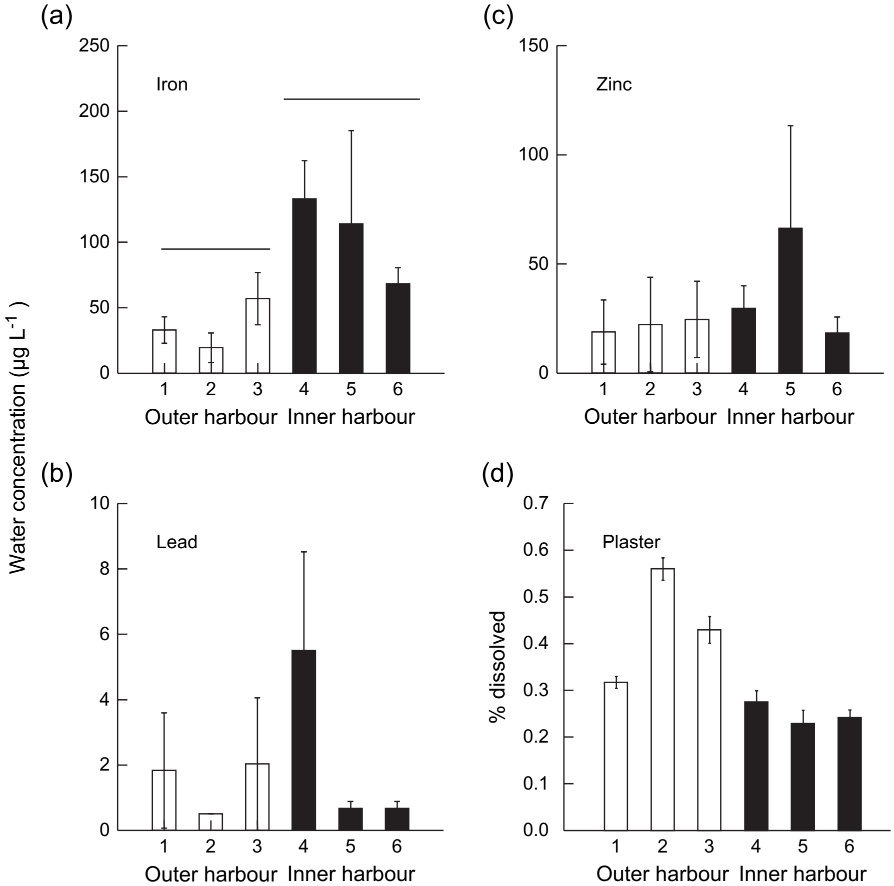

Figure 5. Mean total concentrations of (a) iron, (b) lead, and (c) zinc (μg/L ± 1 SE) in surface water samples ($n = 3$) at sites in Port Kembla Harbor. (d) Proportion of plaster block dissolved over three days at sites in Port Kembla Harbor. Horizontal bars indicate no significant difference between sites.

Water Quality and Pollution

Among the most serious stress factors in estuarine environments are pollution inputs (Kennish 2002). Heavy metals in particular are highly toxic to marine invertebrates interfering with metabolic pathways and binding to sensitive epithelial complexes (Hall et al. 1998). Sites within the Inner Harbor of Port Kembla are historically more metal polluted than those in the Outer Harbor (He & Morrison 2001). As well as suffering higher mortality in the Inner Harbor, our experimental sponges also accumulated more metals particularly at sites closest to Allan's creek and the steelworks. Laboratory studies indicate that sponges accumulate metals in direct proportion to the concentration in the water and retain them upon transfer to clean seawater (Hansen et al. 1995), and they have therefore been recommended as useful biomonitoring tools. A Mediterranean sponge (*Crambe crambe*) collected from a polluted site contained 10 times the metal content (in particular copper) of sponges collected from an uncontaminated site (Cebrian et al. 2003).

Spot water sampling conducted at three times throughout the sponge deployment period produced variable

Table 3. Water quality data from all sites in the Inner and Outer Harbors.

Harbor	*Site*	*pH*	*Salinity (‰)*	*DO (mg/L)*	*Turbidity (NTU)*	*Temperature (°C)*
Outer	1	8.2 ± 0.03	34.6 ± 0.3	7.1 ± 0.4	9.4 ± 3.7	17.5 ± 1.8
Outer	3	8.2 ± 0.01	34.6 ± 0.4	6.9 ± 0.7	8.6 ± 4.0	17.6 ± 1.8
Outer	4	8.2 ± 0.04	34.4 ± 0.4	6.5 ± 1.0	8.7 ± 3.9	17.8 ± 1.7
Inner	5	8.2 ± 0.01	34.8 ± 0.6	6.6 ± 0.6	12.2 ± 4.0	19.5 ± 2.2
Inner	7	8.2 ± 0.02	34.4 ± 0.3	6.6 ± 0.5	9.8 ± 3.7	18.1 ± 1.6
Inner	8	8.2 ± 0.04	34.4 ± 0.5	6.8 ± 0.7	9.4 ± 3.2	18.0 ± 1.6

Measurements were taken on three occasions throughout the experimental period (see Methods). Values are average of the three ± 1 SEs.

Table 4. ANOVA for taxonomic groups in assemblages at six sites within the Inner and Outer Harbors censused prior to the reciprocal transplant.

Taxonomic Group	*Source*	*df*	*Mean Square*	*F*	*p*
Barnacles (√)	Harbor	1	3.126	13.651	**0.021**
	Site (harbor)	4	0.229	2.202	0.131
	Error	12	0.104		
Bare space (ASIN√proportion)	Harbor	1	0.123	7.235	0.055
	Site (harbor)	4	0.017	2.429	0.106
	Error	12	0.007		
Encrusting bryozoans (ASIN√proportion)	Harbor	1	0.001	0.013	0.915
	Site (harbor)	4	0.078	3.391	**0.047**
	Error	12	0.023		
Arborescent bryozoans (√)	Harbor	1	1.196	1.701	0.262
	Site (harbor)	4	0.703	8.369	**0.002**
	Error	12	0.084		
Serpulid polychaetes (√)	Harbor	1	0.028	0.024	0.884
	Site (harbor)	4	1.155	5.226	**0.011**
	Error	12	0.221		
Solitary ascidians (√)	Harbor	1	0.060	0.476	0.528
	Site (harbor)	4	0.126	2.100	0.146
	Error	12	0.060		

Significant differences at $\alpha = 0.05$ are highlighted in bold.

results although iron concentrations were generally higher in the Inner Harbor. This was particularly clear at the two sites closest to the steelworks where iron ore is stockpiled on the harbor bank. In bays and estuaries, pollution inputs are likely to fluctuate through space and time. Industrial inputs, stormwater run-off, and the resuspension of contaminated sediments through shipping or dredging are all likely to contribute sporadically to water column pollution (Beck 1996). Accurate assessment of pollution conditions in these areas may need to be ongoing or almost continuous. In such cases, bioaccumulating organisms, such as sponges, reflect a time-integrated measure of exposure and may be better indicators of water quality than spot water sampling (Hansen 1995).

Other water quality parameters likely to affect the growth and survival of sponges are pH, temperature, salinity, dissolved oxygen, and turbidity. Water quality data collected at the same depth as our experiments indicated that pH, dissolved oxygen, and salinity were relatively constant throughout both harbors. Slightly elevated temperature and turbidity readings occurred at site 5 closest to Allan's creek and the steelwork's water-cooling outlet. Slight elevations in temperature are likely to increase feeding rates, and therefore, the growth of sponges (Riisgard et al. 1993) are thus unlikely to explain our results. Turbidity readings (approximately 10 NTU) were generally reflective of estuarine conditions although again, spot water sampling may have missed sporadic sediment resuspension events. These are likely to be more common in the Inner Harbor where shipping activity is concentrated.

Another potential determinant of sponge growth rate is water flow because higher flow may result in a higher delivery of food particles to these suspension-feeding organisms (Bell 2002). It is difficult to model flow rates on a highly localized scale, so we used the common technique of measuring plaster block dissolution rates to give a relative measure of flow between sites (Thompson & Glenn 1994). Block dissolution was site specific, indicating higher flow rates at two sites in the Outer Harbor and reduced flow rates at four other experimental sites. The highest sponge survival and growth occurred at site 1 in the Outer Harbor which recorded reduced flow conditions similar to those of the Inner Harbor. Flow rates alone are therefore unlikely to account for differences in sponge survival and growth. Measures of food availability (ultraplankton counts) within the water column would be useful to further assess the role of flow in influencing our results (Pile et al. 1996).

The positive role of stationary filter feeders such as sponges influencing water quality may ironically make them more susceptible to poor water quality and pollution due to the large volumes of water passing through them. The restorative services of these organisms are only likely to operate within an upper range of water quality, and once a threshold is breached, physical restoration through biological means may no longer be possible. This compounds the problem, leading to further deterioration of water quality and rapid loss of local biota. Physical remediation would then require large-scale cleanup projects, which are notoriously expensive and of varying success (Kelaher et al. 2003). Preserving environmental conditions that facilitate the removal of contaminants by biological agents is desirable on both ecological and financial grounds and should be the primary strategy employed by environmental managers.

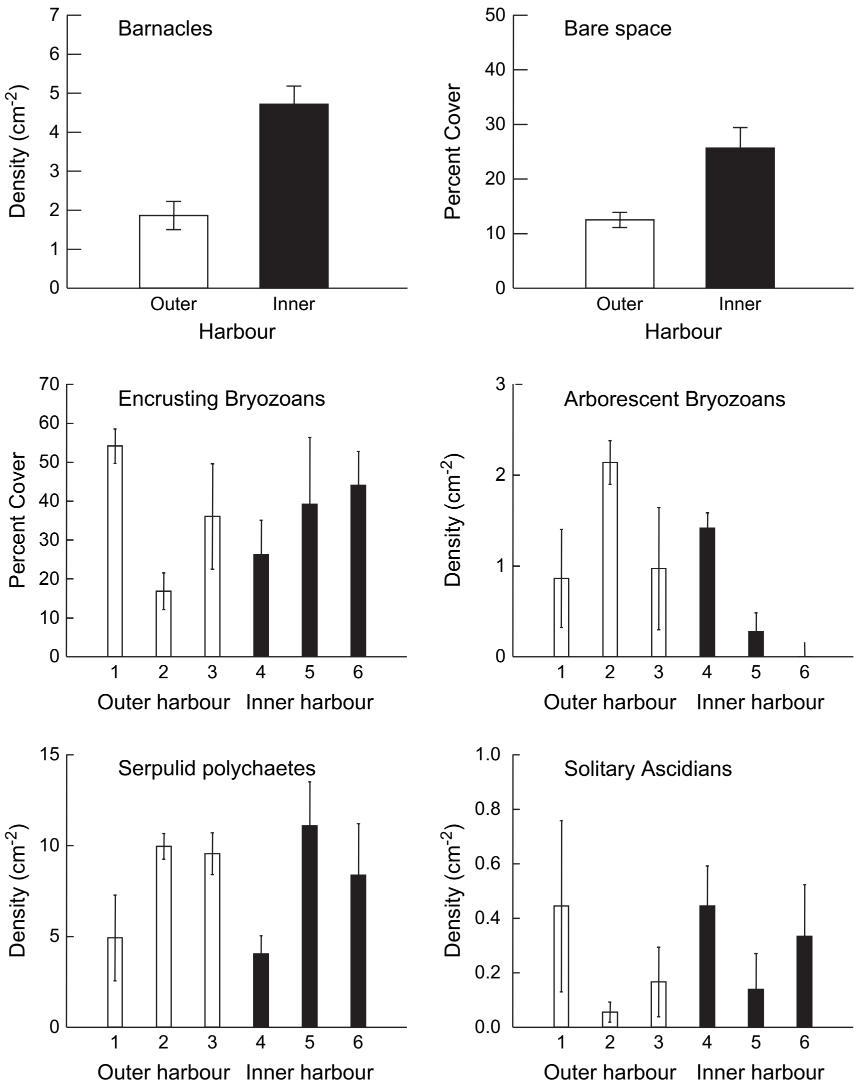

Figure 6. Initial assemblage composition at all six sites within Port Kembla Harbor prior to sponge deployment and the reciprocal transplant. Where ANOVA found no difference at the level of site, data are presented within Inner and Outer Harbors only (barnacle density and percent cover of bare space). Where there were differences at the site level, data are presented for each individual site (encrusting and arborescent bryozoans, serpulid polychaetes, and solitary ascidians).

Community Composition

Community composition and competition have been shown to influence the success of several transplanted species (e.g., grasses; Carlsen et al. 2000) and should be an important consideration in any trial species reintroduction. Community composition is known to affect the survivorship of organisms in hard-substrate marine assemblages dominated by competition for space (Connell & Keough 1985; Buss 1986). Sponges are generally poor recruiters but good competitors for space, capable of overgrowing early colonizing organisms such as serpulids, barnacles, and bryozoans (Jackson 1977; Kay & Keough 1981). We would therefore predict high survival rates of sponge colonies that were introduced into hard-substrate assemblages not already dominated by good competitors such as ascidians or other sponges.

The initial experimental assemblages grown for five months in Port Kembla Harbor differed between sites. Overall, they might be characterized as highly disturbed assemblages dominated by early colonizing species such as barnacles and serpulids (Johnston & Keough 2005). Settlement panel assemblages established in less disturbed bays and estuaries are often dominated by sponges and ascidians within a four-month recruitment period (Johnston & Keough 2002; Johnston et al. 2002), and by the end of the sponge deployment period, experimental assemblages at two Outer Harbor sites more closely resembled such undisturbed communities. Inner Harbor assemblages retained a distinctly disturbed composition. On the basis of community composition alone, we would predict a greater success of sponge transplants in assemblages that had

Table 5. ANOVA for taxonomic groups in assemblages at six sites within Port Kembla Harbor censused at the end of the experiment.

Taxonomic Group	*Source*	*df*	*Mean Square*	*F*	*p*
Colonial ascidians	IH	1	0.006	0.189	—
	FH	1	1.512	45.796	—
	IH × FH	1	0.060	1.809	—
	IS (IH)	4	0.031	0.944	0.444
	FS (FH)	4	0.173	5.229	**0.001**
	FH × IS (IH)	4	0.025	0.751	0.561
	IH × FS (FH)	4	0.010	0.315	0.867
	FS (FH) × IS (IH)	16	0.014	0.410	0.976
	Error	72	0.033		
Bare space	IH	1	0.181	7.593	—
	FH	1	1.618	67.929	—
	IH × FH	1	0.000	0.000	—
	IS (IH)	4	0.022	0.932	0.450
	FS (FH)	4	0.259	10.856	**0.000**
	FH × IS (IH)	4	0.026	1.091	0.367
	IH × FS (FH)	4	0.022	0.919	0.458
	FS (FH) × IS (IH)	16	0.018	0.751	0.733
	Error	72	0.024		
Serpulids	IH	1	0.123	8.238	—
	FH	1	0.030	2.014	—
	IH × FH	1	0.019	1.289	—
	IS (IH)	4	0.037	2.505	**0.050**
	FS (FH)	4	0.072	4.819	**0.002**
	FH × IS (IH)	4	0.027	1.789	0.140
	IH × FS (FH)	4	0.026	1.742	0.150
	FS (FH) × IS (IH)	16	0.022	1.489	0.128
	Error	72	0.015		
Barnacles	IH	1	0.067	15.628	—
	FH	1	0.010	2.353	—
	IH × FH	1	0.012	2.729	—
	IS (IH)	4	0.010	2.319	0.065
	FS (FH)	4	0.017	3.915	**0.006**
	FH × IS (IH)	4	0.007	1.661	0.168
	IH × FS (FH)	4	0.005	1.199	0.319
	FS (FH) × IS (IH)	16	0.004	0.921	0.549
	Error	72	0.004		
Solitary ascidians	IH	1	0.025	5.025	—
	FH	1	0.018	3.662	—
	IH × FH	1	0.004	0.864	—
	IS (IH)	4	0.016	3.169	**0.019**
	FS (FH)	4	0.004	0.727	0.576
	FH × IS (IH)	4	0.001	0.263	0.901
	IH × FS (FH)	4	0.002	0.359	0.837
	FS (FH) × IS (IH)	16	0.003	0.552	0.908
	Error	72	0.005		
Encrusting bryozoans	IH	1	0.004	0.714	—
	FH	1	0.009	1.422	—
	IH × FH	1	0.009	1.422	—
	IS (IH)	4	0.019	3.136	**0.020**
	FS (FH)	4	0.010	1.627	0.177
	FH × IS (IH)	4	0.008	1.407	0.240
	IH × FS (FH)	4	0.009	1.427	0.234
	FS (FH) × IS (IH)	16	0.006	1.034	0.433
	Error	72	0.006		

"Initial" and "Final" refer to positions held prior to, and post, the reciprocal transplant. There is no suitable *F* test for the first three factors due to the presence of two random factors in the analysis (Downes et al. 2002). Brackets indicate nesting, and significant differences at $\alpha = 0.05$ are highlighted in bold. IH, Initial Harbor; FH, Final Harbor; IS, initial site; FS, final site.

developed within the Inner Harbor, because there was more available space and less competition. However, we found no effects of initial community composition on sponge growth, and the influence of recipient environments may have masked the effects of community composition. Inner Harbor conditions appear to be chronically

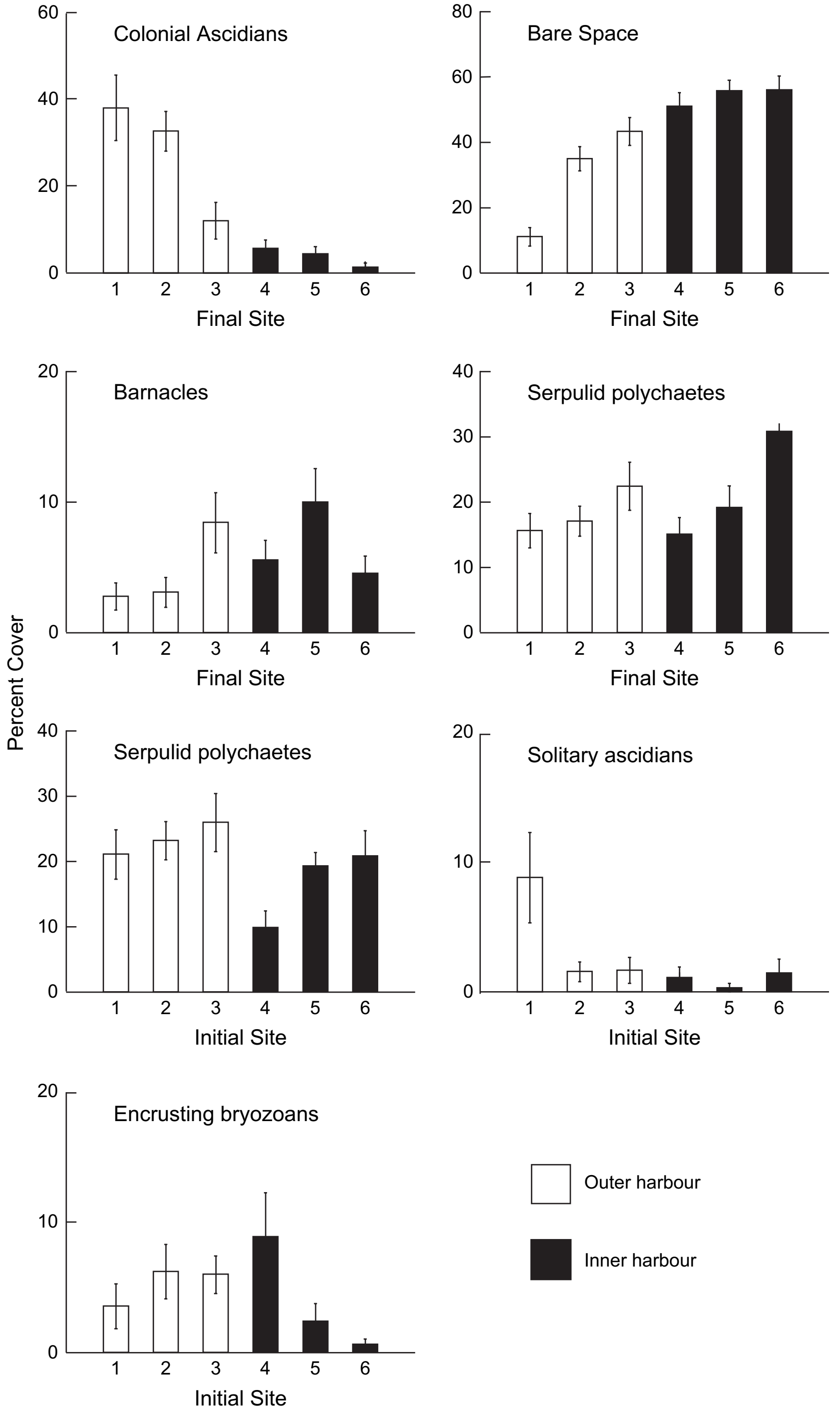

Figure 7. Final invertebrate assemblage composition at all six sites within Port Kembla Harbor following sponge deployment and reciprocal transplant. Where ANOVA found a difference attributable to the final site position, data are presented for this factor (colonial ascidians, bare space, barnacles, and serpulid polychaetes). Where there were differences attributable to the initial site position, data are presented for this factor (serpulid polychaetes, solitary ascidians, and encrusting bryozoans). All data are presented as percent cover of invertebrate.

or frequently disturbed in a way that permanently prevents the establishment of good competitors.

Although sponges at some sites faired better than others, most sponges decreased in surface area over the study period. This may be expected because the study was conducted during winter, and colder temperatures typically retard growth rates (Bell 2002). It may also be attributed to stress incurred during the transplant procedure, although sponges were handled with care and were only exposed to air for very brief periods (<10 seconds). Alternatively, it may reflect the overall water quality of the port, which, while variable between harbors, is still poorer than the site from which the sponges were originally collected (Botany Bay). These factors could be better understood by running the study for a longer period over multiple seasons and assessing the ongoing survival, reproduction, and persistence of the species. A full-scale reintroduction would also need to consider the genetic variability of explants because genetic diversity may reduce the likelihood of failure due to natural population fluctuations (Williams & Davis 2004).

Conclusions

Restoration projects are often required to achieve specific goals, usually related to reintroduction of key species or entire assemblages. Failed attempts may be later attributed to historical or ongoing environmental conditions such as groundwater supply or sedimentation (Bond & Lake 2003). Due to the high expenses involved in restoration projects, there is a need for smaller pilot or trial studies that first identify physical, chemical, or biological barriers to successful reintroductions. This will also allow us to identify requirements for restoring a system that is ultimately self-structuring and one where persistence and reproduction of organisms are likely.

The decision about where to attempt a restoration project is often based on an assessment of the conditions required to sustain the restoration over a long time (Peterson & Lipcius 2003). This requires an assessment and prediction of the environmental and biological conditions necessary for success. The temporal scale of our study was not sufficient to assess the longer-term sustainability of the sponge reintroduction, but the evidence strongly suggests that sponge transplant attempts would *not* be a useful strategy for restoring the biological integrity of assemblages within Port Kembla Harbor. Remediation of the chemical environment may be a prerequisite for biological remediation and this will not be achievable without massive intervention.

Implications for Practice

- Pilot studies are useful in identifying physical, chemical, and biological barriers to successful reintroductions.
- Success of sponge reestablishment will strongly depend on recipient environmental conditions.
- Heavy-metal pollution and/or suspended sediment may limit the success of marine restorations involving sessile filter feeders.
- Composition of sessile invertebrate community has little influence on sponge transplant success.

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