Coastal upwelling may overwhelm the effect of sewage discharges in rocky intertidal communities of the Peruvian coast

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Abstract. In coastal productive environments, such as upwelling systems, sewage effluents might represent an important input of nutrients affecting intertidal community development and structure. Using descriptive and experimental approaches, the community spatial variation and early succession in relation to a point-source effluent discharge in a rocky intertidal of an upwelling affected area (Ancón Bay, Peru) was analysed. The relative contribution of herbivory to the observed patterns was also analysed. Dissolved nutrient concentrations, macroalgal isotopic signatures and N content revealed a significant input of nutrients at the outfall, although this contribution was not reflected in the algal assemblage, but in higher abundances of mytilids, ophiuroids and limpets. Cover of most sessile organisms (biofilm, *Ulva* spp., mytilids and barnacles) varied among sites throughout early succession, and grazers only enhanced the cover of the red algae *Gelidium* spp. Differences in succession patterns could not be attributed to discharge effects. The results of this study suggest that the community development is bottom-up controlled in the entire bay, which is likely to be due to the upwelling that operates at larger temporal and spatial scales. If so, nutrient input derived from coastal upwelling may sometimes overwhelm the role of anthropogenic nutrient loadings in shaping intertidal communities.

Additional keywords: bottom-up, community structure, Peru, succession.

Introduction

The structure and dynamics of communities and ecosystems are determined by many ecological processes such as biological interactions (e.g. predation, competition) and primary production (e.g. Menge 1992). Consumers frequently have important effects on the structure and dynamics of benthic marine systems (Wootton 2002), influencing the distribution, diversity and abundance of faunal and macroalgal assemblages on shores of varying exposures worldwide (reviewed by Hawkins and Hartnoll 1983; Williams *et al*. 2000). Moreover, there is increasing information showing that nutrients also play an important role in structuring coastal communities (e.g. Valiela 2006). Nutrient availability, in particular nitrogen and phosphorus, which are considered to be the limiting nutrients in most marine ecosystems (Valiela *et al*. 1997), can exert a bottom-up control on lower trophic levels, propagating up through the trophic webs influencing plant–herbivore interactions and abundances (Menge 1992; Nielsen and Navarrete 2004).

Factors operating at different temporal and geographical scales, such as global climate change, variability in local currents, upwellings and coastal eutrophication, can alter nutrient concentrations in coastal environments (e.g. Valiela 2006). In coastal upwelling systems, surface waters are displaced offshore by a one-sided horizontal divergence caused by surface Ekman transport and are replaced by nutrient-rich deep water (Pennington *et al*. 2006). Among other effects, upwellings can modify the abundance of some macroalgal functional groups. For instance, in the proximity of south-eastern Pacific upwelling centres, turf-forming and corticated algae grow faster, and attain taller heights and higher biomass (Nielsen and Navarrete 2004; Wieters 2005), which results in lower abundance of ephemeral algae (Broitman *et al*. 2001). However, higher densities of filter-feeders and herbivores have not been associated with stronger upwelling (Broitman *et al*. 2001), while the latter play a minor role in macroalgal control (Nielsen and Navarrete 2004). Thus, bottom-up and top-down forces simultaneously influence

macrobenthic populations and communities (e.g. Menge 1992). Their relative strengths, however, may vary with environmental conditions (Lotze *et al.* 2001).

At a smaller scale, sewage effluents are one of the most common anthropogenic disturbances of near-shore marine benthic communities and have been recognised as one of the main causes of their assemblage changes (e.g. Valiela 2006). Their impacts on intertidal organisms often follow gradients in the concentration of contaminants (Bishop *et al.* 2002), producing assemblages dominated by few species (e.g. Littler and Murray 1975), by reducing species richness and diversity (López Gappa *et al.* 1990; Valiela *et al.* 1997), or increasing the recruitment and productivity of opportunistic organisms such as ephemeral green algae, holding the community at an early successional stage (Fairweather 1990). Effluents may also cause habitat modification (Wear and Tanner 2007) and, in some cases, increase variability (e.g. Warwick and Clarke 1993). These biological consequences of sewage disposal have been widely reported, and some features can be considered widespread (e.g. Valiela 2006). In some cases, however, natural variations of the marine environment seem to be more influential than the source of impact in shaping benthic communities (see Wear and Tanner 2007).

The central Peruvian Ancón Bay is located within a main upwelling centre, receiving waters with high nutrient concentrations and low oxygen content (Tarazona *et al.* 2003). In addition, there is an input of anthropogenic-derived nutrient through outfalls that discharge urban raw sewage into the bay. Thus, based on the current knowledge, we hypothesised that even in such a productive system, wastewater discharge would produce a gradient in nutrient concentration associated with a decreasing cover of ephemeral algae and higher abundances of filter-feeders and grazers with increasing distance from the point of discharge.

Materials and methods

Study site

The study was conducted on the rocky shore south of Ancón Bay (11°46S, 77°11W, 50 km north of Lima, Peru). The regional climate is characteristic of the Peruvian arid coastal desert, with an average temperature of 19.5°C and 12 mm of rain per year (Galán de Mera *et al.* 2004). Ancón, visited by thousands of tourists during summer, contributes through domestic sewage, the only source of anthropogenic nutrient input. The upwelling-derived waters responsible for the permanent hypoxic conditions that prevail close to the seafloor, also characterise Ancón Bay (~0.5 mL O_2 L^{-1}, Tarazona *et al.* 1988, 2003). Since these climatic, demographic and oceanographic features are common to other bays of the region, Ancón can be considered as representative of many Peruvian central coast bays.

Four sites were selected in the bay: Desague (Des; 11°46′14S, 77°11′19W) where untreated urban effluent is discharged through a secondary outfall directly to the shore with a mean flow rate of 1150 m³ day⁻¹ (E. Ramos, pers. comm.), Punta Cruz (PC; 11°46′12S, 77°11′27W), Playa Hermosa (PH; 11°46′26S, 77°11′14W) and Muelle San Martin (SM; 11°46′32S, 77°10′49W). PC and PH are 250 m west and east, respectively, from the outfall, and SM 700 m west (Fig. 1). Whereas Des and PC are natural rocky shores, PH and SM are both seawalls made of the same granite rock that makes up the
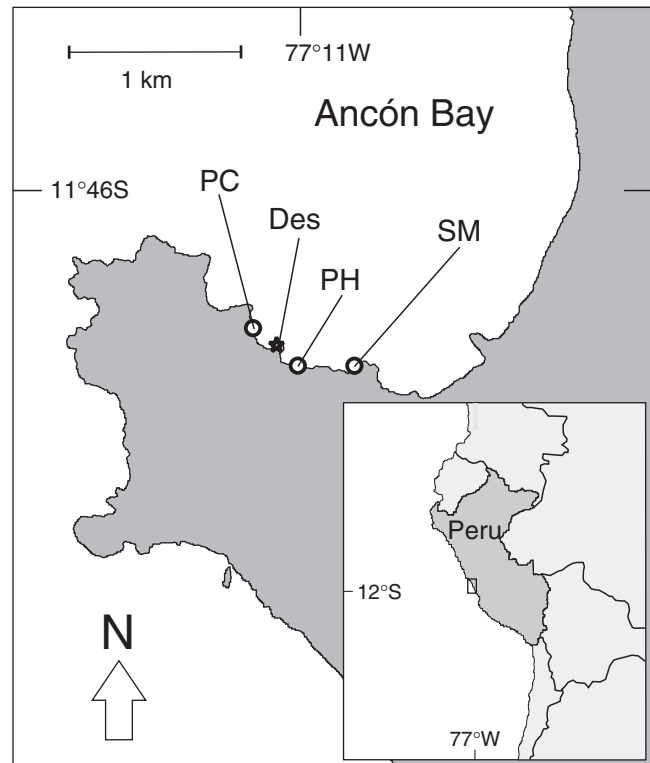

Fig. 1. Map of the study sites: Punta Cruz (PC), Desague (Des), Playa Hermosa (PH) and San Martin (SM).

natural rocky reefs in this area; furthermore, both structures have similar slopes and exposure to waves.

Assessment of dissolved nutrients

To evaluate the nutrient concentrations, water samples were collected weekly from the surf zone from November 2005 to January 2006 (late austral spring–early summer, when sewage discharge would be more intense due to tourism). Three replicated seawater samples were collected, filtered (50 μm), stored in 250-mL polyethylene bottles and frozen at −20°C until processing. We then quantified phosphate (Murphy and Riley method), silicate (Mullin and Riley method), nitrate (Morris and Riley method) and nitrite (Bendschneider and Robinson method; all methods described in Strickland and Parsons 1972). Given logistic constraints, we were unable to measure the highly volatile ammonium.

Differences in nutrient concentrations among sites and weeks were analysed with two-way ANOVAs. When interactions between two or more factors were significant, Tukey's honestly significant difference (HSD) tests were used to identify how factors interacted (Zar 1999). Data were not transformed given that the ANOVA is robust to heterogeneous variances when sampling is balanced (see Underwood 1997).

Ulva spp., an opportunistic macroalga, was used to infer nutrient supply through tissue nitrogen content and stable isotope analysis (McClelland and Valiela 1998). Macroalgae have the advantage of integrating the relatively long-term effects of small or pulsed nutrient inputs, and are able to detect pulses of

N that are not measurable by short-term sampling, thereby serving as a reliable indicator of environmental conditions (Cohen and Fong 2006; Lin *et al*. 2007). *Ulva* fronds of approximately the same size were collected in May 2006 from each study site. These samples were rinsed with distilled water, oven-dried at 60°C for at least 48 h and then ground to a fine powder. Tissue C and N content and stable isotope signature for $\delta^{15}N$ were determined by mass spectrometry (see Lajtha and Michener 1994; for details). Differences in measured variables among sites were analysed with one-way ANOVAs with subsequent Tukey's HSD tests (Zar 1999).

Community structure

The specific composition and relative abundance of intertidal benthic organisms at Ancón Bay were determined in October 2006. At each study site, 10 quadrats of 10×10 cm, haphazardly placed on the mytilid mid-intertidal belt, were sampled. All organisms were removed by scraping. Samples were then sieved through a 1-mm mesh and all organisms were identified to the lowest possible taxonomic level (usually genus or species). The animals were counted and algal dry mass was determined by drying at 80°C to constant weight.

To compare assemblages in the different sites of the bay, two separate sets of multivariate and univariate analyses were performed, one for macrofauna with abundance data and the other for algae with biomass data. Bray–Curtis similarity matrices using untransformed data were calculated and non-metric Multidimensional Scaling (nMDS) ordinations were used to provide visual representations of sample similarities (Clarke and Warwick 2001). One-way analyses of similarity (ANOSIM) were performed (Clarke 1993) on all replicates to test for differences among sites. The percentage contribution of each taxon to patterns of dissimilarity between locations was calculated (Clarke 1993). Taxa contributing at least 10% of dissimilarity were considered important differentiators (Bulleri 2005). The abundances of these taxa were analysed separately by one-way ANOVAs, followed by Tukey's *post hoc* tests. Data were transformed to $X^{0.25}$ (similar to log-transformations but used when there are many zeroes; Bulleri *et al*. 2005) to increase homogeneity of variance and normality. Separate one-way ANOVAs with Tukey's *post hoc* tests were used to explore spatial differences among sites in abundances of the most conspicuous grazers.

Succession

To study the relative effects of nutrient availability and grazing in shaping community structure at the study sites, we performed an early succession experiment between November 2005 and December 2006. Eighteen 12×12-cm plots were cleared in the mid-intertidal, with the only restriction that adjacent plots were separated by at least 1 m. Plots were cleared by scraping the substratum to remove all foliose macro-algae, sessile animals and most encrusting algae. At each site, six replicate plots were randomly assigned to each of three treatments: complete herbivore exclusion, half barrier (as procedural control) and un-manipulated open area. Complete exclusions were made by setting a barrier of epoxy putty (Adhesivos Parsecs S. A., Buenos Aires, Argentina) 2 cm wide and painted with antifouling

paint forming a 10×10-cm plot on the cleared rock. The procedural controls were L-shaped partial barriers (Williams *et al*. 2000), and the un-manipulated plots had the corners marked with epoxy putty. As previous studies employing the copper-based antifouling paint have reported little or no effect from the copper on algal recruitment or growth, this method is now widely used to exclude molluscan grazers in intertidal studies (e.g. Nielsen 2001). Although this method potentially allows access to isopods, amphipods and other mesograzers, antifouling prevents highly effective grazers like most limpets and chitons from entering the experimental areas (Hidalgo *et al*. 2008) and can be considered valid for evaluating herbivory effects. The percentage of cover for sessile invertebrates and algae and the density of motile animals were determined using digital photographs in September, March and December 2006. For this purpose, we used a digital grid with 64 evenly spaced dots covering the central 8×8-cm area of the plots, avoiding edge effects. Percentage of coverage was expressed as the number of dots overlying each species. A change in the rock colour (usually green) was scored as 'biofilm' (see Williams *et al*. 2000). Some algae and invertebrates (e.g. small limpets and mytilids) could not be identified to species level and were combined into larger groups (Foster *et al*. 2003).

For statistical comparisons, the six most abundant groups, which occupied more than 80% of the total available space, were selected: the green algae *Ulva* spp., the red algae *Gelidium* spp., the barnacle *Jehlius cirratus*, the mytilids *Perumytilus purpuratus* and *Semimytilus algosus*, and bare space.

As the data for successive measures of each plot were not independent, repeated-measures ANOVAs were used to test the effects of site, treatment and temporal variation on species cover, with site and treatment (between subjects) as fixed factors. Time was analysed as a within-subject effect. In cases of departure of sphericity in the repeated-measures factors (Mauchley's Sphericity Test), we performed the Greenhouse–Geisser Epsilon (GGE) adjustment of the degrees of freedom. Normality was assessed using the Shapiro-Wilk test (Zar 1999). Most data were normally distributed and variances were either homogeneous or nearly so after arcsine square-root transformation. When interactions between factors were significant, multiple comparisons Tukey's *post hoc* tests were used to identify how factors interacted (Zar 1999).

Results

Assessment of dissolved nutrients

Nitrate, nitrite, silicate and phosphate concentrations in the water were highly variable over time and among sites ($F_{24,72} = 5.12$, 3.39, 1.98 and 4.15, respectively, $P < 0.001$ for all tests except for silicate, $P = 0.014$). Nitrate showed a clear spatial and temporal pattern, with a peak of highest concentration at Des during the first 3 weeks (Tukey's HSD test, $P < 0.05$; Fig. 2). Nitrite showed a similar pattern, but peaked at Des only the second week along with silicate (Fig. 2). A peak in phosphate was observed at PH the sixth week; however, the concentration was not different from Des. No further differences among sites at any given time were found (Tukey's HSD test, $P > 0.05$; Fig. 2).

Stable isotopic signatures, tissue N content and C : N ratios of macroalgae differed among sites. Signatures and C : N
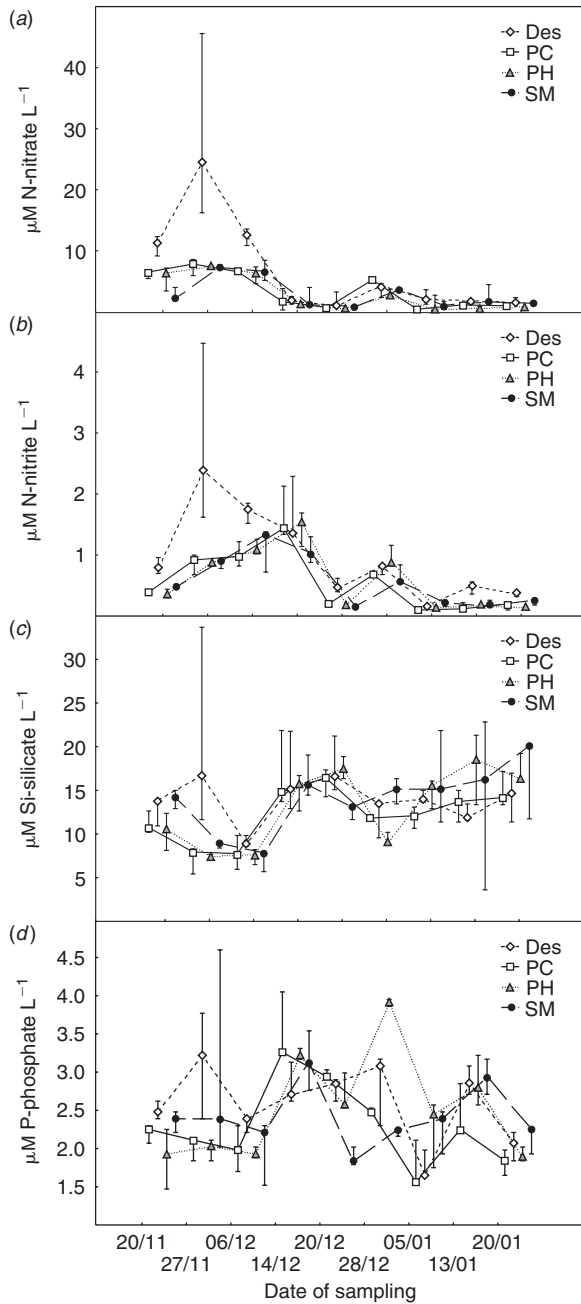
Fig. 2. Weekly sampling of (*a*) surf-zone nitrate, (*b*) nitrite, (*c*) silicate and (*d*) phosphate at Ancón Bay (median, 5th to 95th percentiles). First 6 weeks correspond to 2005 and the last 3 weeks correspond to 2006. Punta Cruz (PC), Desague (Des), Playa Hermosa (PH), San Martin (SM).

Fig. 3. N content, C : N ratios and $\delta^{15}N$ signatures of *Ulva* fronds at the study sites (median; box, 25th to 75th percentiles; whiskers, 5th to 95th percentiles). Different letters denote significant differences. Punta Cruz (PC), Desague (Des), Playa Hermosa (PH), San Martin (SM).

ratios of *Ulva* growing at Des were lower than all other sites ($F_{3,16} = 197.8$ and 14.3, respectively, $P < 0.001$ for both tests; Fig. 3). Furthermore, tissue N in fronds from Des showed the highest values ($F_{3,16} = 41.8$, $P < 0.001$; Fig. 3).

Community structure

A total of 51 species were sampled. Within the invertebrates, Mollusca was the most taxon-rich group, followed by Annelida.
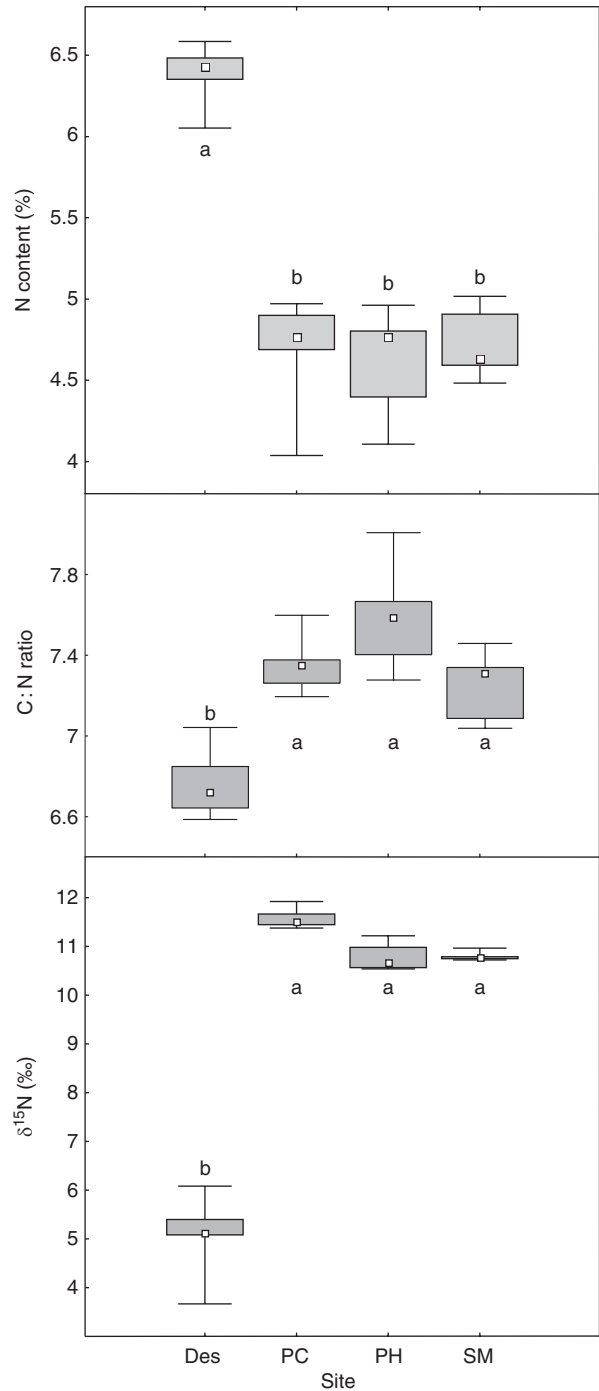
Algae were represented by two species of Chlorophyta (*Ulva* and *Bryopsis* spp.), and six of Rhodophyta (*Gelidium*, *Grateloupia* spp., *Polysiphonia* spp., *Chondracanthus chamissoi*, *Gracilariopsis* spp. and *Ceramium* spp.).

The analysis of similarity revealed differences in structure of macrofauna assemblages among sites ($R = 0.39$, $P < 0.001$;

Fig. 4), and pairwise tests showed differences for all possible pairs of comparisons between sites ($P < 0.05$ for all tests). In contrast, algal assemblages did not differ among sites ($R = 0.074$, $P = 0.075$; Fig. 4). At all sites, *Ulva* and *Chondracanthus chamissoi* were the predominant algae. Only four taxa could explain much of the dissimilarity among sites. The mytilids *Perumytilus purpuratus* and *Semimytilus algosus*, the ophiuroid *Ophiactis* spp. and the polychaete *Pseudonereis* spp. distinguished consistently among locations (Table 1).

The mussel *Perumytilus purpuratus* was most abundant at Des, and different from PC and SM; the lowest abundance was at SM, and differed from PH ($F_{3,36} = 8.25$, $P < 0.001$; Tukey's HSD test, $P < 0.05$; Fig. 5). Abundance of the mussel *S. algosus* at Des was higher than at PH (Fig. 5). At Des and PH, the ophiuroid *Ophiacti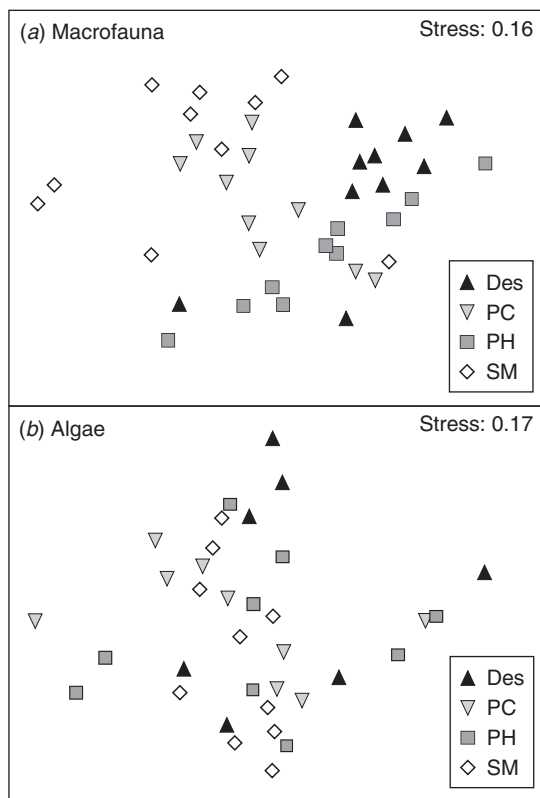s* spp. abundances were higher than at PC and SM ($F_{3,35} = 23.15$, $P < 0.001$; Fig. 5). The lowest abundance of the polychaete *Pseudonereis* spp. was at SM ($F_{3,36} = 8.6$, $P < 0.001$; Fig. 5). The most abundant molluscan grazer was the limpet *Scurria* spp., followed by juvenile chitons and *Prisogaster niger*, whereas the most conspicuous mesograzers were isopods. The limpets *Scurria* spp. and *Siphonaria* spp. were both more abundant at Des ($F_{3,36} = 6.3$ and 3.7, respectively, $P = 0.001$ and 0.02, respectively; Fig. 6).

Succession

Twenty species were identified in succession samplings. All algae settled within the 4 months after plots were cleared and persisted during the year, except the Rhodophyta *Grateloupia* spp., which colonised later, starting from September, and *Polysiphonia* spp., which disappeared after March. The barnacle *Balanus laevis*, the sea anemone *Anthothoe chilensis* and the polychaete *Phragmatopoma moerchi* also settled starting from September. Only biofilm and the red algae *Gelidium* were affected by herbivores. The interaction site × herbivory was significant for biofilm ($F_{6,43} = 2.76$, $P = 0.02$). Cover in exclusions at SM was higher than in exclusions at Des and PC, controls at Des, PH and PC, and in un-manipulated areas at PH (Tukey's HSD test, $P < 0.05$; Fig. 7). Biofilm cover was lowest in September and increased in December ($F_{1.5,63.8} = 26.7$, $P < 0.001$; Fig. 7). Grazing effects on *Gelidium* were positive ($F_{2,43} = 4.5$, $P = 0.01$). Interestingly, at most sites and on most dates, *Gelidium* cover was higher at non-manipulated areas than in exclusions and controls (Tukey's HSD test, $P < 0.05$; Fig. 7).

For *Ulva*, the interaction site × time was significant ($F_{6,86} = 4.75$, $P < 0.001$). *Ulva* cover peaked at SM during September, and was different from March and September at Des and PC, from March and December at PH, and from March at SM. Cover in December at SM was higher than in September at Des and March at PH. Finally, cover in December at Des was higher than in March at PH (Tukey's HSD test, $P < 0.05$; Fig. 7).

Mytilid cover varied among sites and time ($F_{3,43} = 14.55$, $P < 0.001$ and $F_{2,86} = 5.19$, $P = 0.007$, respectively). Cover at PC was higher than at all other sites, and Des was different from SM, with the lowest cover. Mytilids reached maximum cover during September, which was higher than March (Tukey's HSD test, $P < 0.05$; Fig. 7).

Jehlius cirratus cover also differed among sites and time ($F_{3,43} = 8.1$, $P < 0.001$ and $F_{2,86} = 10.15$, $P < 0.001$, respectively). Cover was greatest at PH and PC and exceeded Des and SM covers. This species reached maximum cover in March and decreased in September and December (Tukey's HSD test, $P < 0.05$; Fig. 7). Bare space varied among sites ($F_{3,43} = 5.63$, $P = 0.002$); the lowest amount of unoccupied

Fig. 4. Non-metric multidimensional scaling (nMDS) plots on untransformed data comparing (*a*) macrofauna and (*b*) algae assemblages at the study sites. Punta Cruz (PC), Desague (Des), Playa Hermosa (PH), San Martin (SM).

Table 1. Percentage contribution of each macrofaunal taxon (contributing >10% at least once) to the dissimilarity in assemblage structure between sites within Ancón Bay

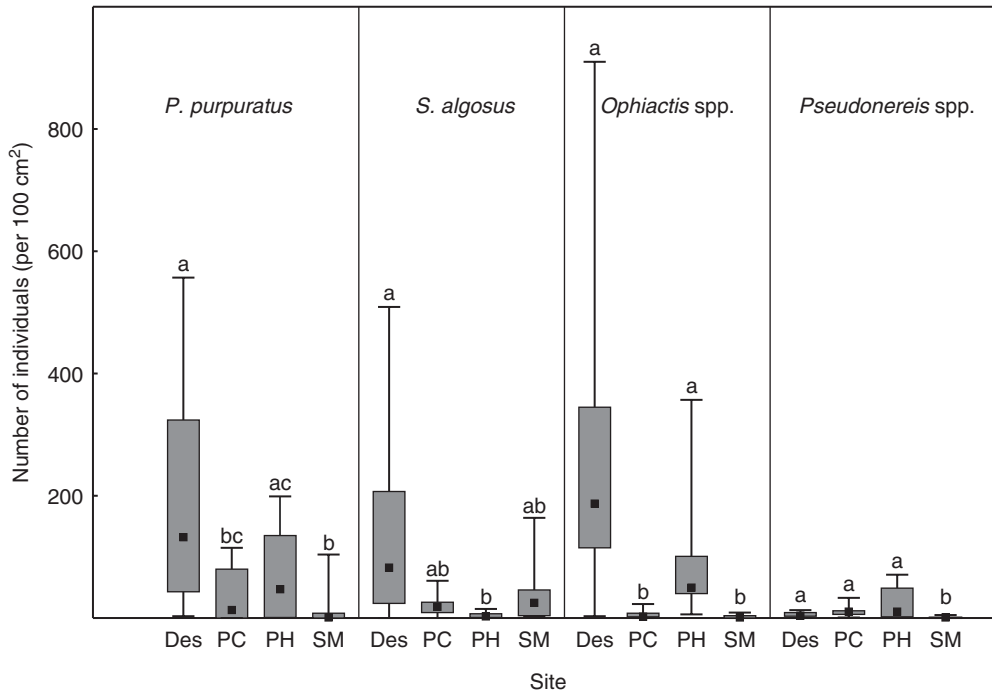| Taxa	Des–PC	Des–PH	PC–PH	Des–SM	PC–SM	PH–SM
S. algosus	27.86	25.19	20.83	28.04	27.07	17.14
P. purpuratus	16.20	14.31	–	18.06	23.51	14.97
Ophiactis spp.	31.35	34.10	37.14	31.23	–	37.66
Pseudonereis spp.	–	–	11.58	–	–	13.67

Fig. 5. Abundance of the main species differentiating among sites at Ancón Bay. Different letters denote significant differences. Punta Cruz (PC), Desague (Des), Playa Hermosa (PH), San Martin (SM).
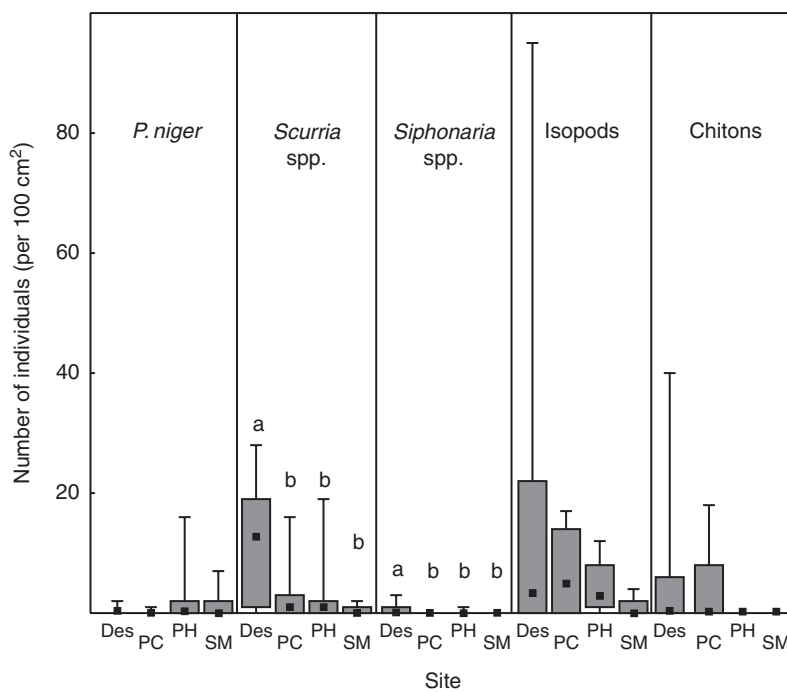

Fig. 6. Abundance of the most abundant grazers at Ancón Bay. Different letters denote significant differences. Punta Cruz (PC), Desague (Des), Playa Hermosa (PH), San Martin (SM).
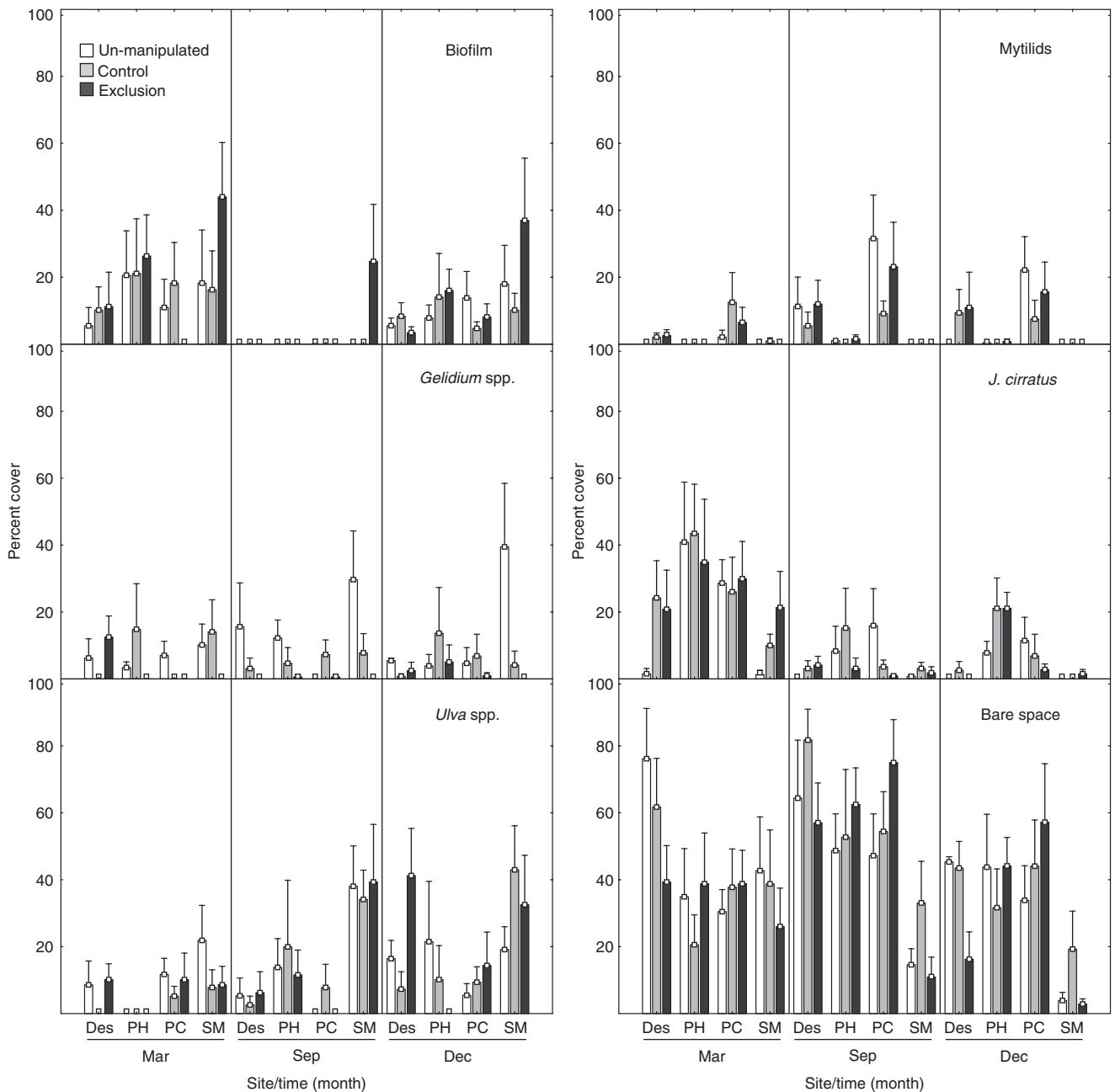
Fig. 7. Percentage of cover of the most abundant groups at each study site during succession. White boxes represent un-manipulated plots, light grey boxes are experimental control plots and dark grey boxes show exclusion plots (mean; +s.e.).

space was observed at SM, which was different from PC and Des, with the highest bare space (Tukey's HSD test, $P < 0.05$; Fig. 7).

Discussion

Nutrient pulses and higher algal nitrogen content were observed at the outfall of Ancón Bay. At the local scale, differences in nutrient loading among sites were not reflected in algal assemblages; however, the abundances of most conspicuous invertebrates were higher at the discharge point. During succession, temporal and spatial variation in the cover of sessile organisms showed no consistent pattern in relation to the outfall distance and only one macroalgae species was affected by grazers, increasing its cover. Moreover, among-site differences could not be attributed to discharge effects.

Most studies demonstrating the impacts of nutrients on primary production and benthic diversity in upwelling regions have dealt with the influence of seabird guano (Bosman and Hockey 1986; Bosman *et al.* 1986), or with the experimental addition of nutrients (Nielsen 2001). Comparison among these studies is hindered by differences in the kinds of reported data, and

by methodological differences (Nielsen 2001). Domestic waste effects have been studied mainly in oligotrophic systems, reporting changes in the community structure at the scale of hundreds of metres (e.g. López-Gappa *et al.* 1990; Smith 1996; Bishop *et al.* 2002). Thus, our work provides new insight on this issue.

Assessment of dissolved nutrients

Nitrate concentrations in Ancón Bay ranged from 0.2 to 45.6 μmol L^{-1}, and were more variable than previously reported for the Peruvian central coast in summer (from 3 to 5 μmol L^{-1}, Graco *et al.* 2007) and for other upwelling systems (e.g. 1–15 μmol L^{-1} in South Africa, Bustamante *et al.* 1995; 1–10 μmol L^{-1} on the western coast of the South Island of New Zealand, Menge *et al.* 1999). Silicate reached the highest concentration at Des, probably related to freshwater inputs (see Cox *et al.* 2006). Phosphate was highly variable, and did not show any apparent trend. The decoupling between nitrate and phosphate concentrations has been observed when related to periodic episodes of freshwater loading (Cox *et al.* 2006). Furthermore, in this oxygen-depleted system, nitrogen becomes exhausted before phosphate due to denitrification (Pennington *et al.* 2006). The increased nitrate, nitrite and silicate concentrations observed at Des suggest a direct input of wastewater. Since in sewage spills an abundant nitrogen form is ammonia (Oczkowski *et al.* 2008), the lack of ammonia measures may have led us to underestimate the differences in nitrogen concentration among sites.

The lowest *Ulva* δ^{15}N values observed at the outfall ($\sim$5‰) are typical of untreated wastewater (5 to 9‰, Cole *et al.* 2006). Although algal δ^{15}N values usually decrease with distance to outfalls in sewage-affected sites (Savage and Elmgren 2004; Lin *et al.* 2007), upwelling in zones of high denitrification can elevate δ^{15}N values to +10–12‰ (Liu and Kaplan 1989), resembling the values found at PC, PH and SM. A higher N content and lower C : N ratio were detected in *Ulva* growing at Des, although at all sites *Ulva* reached N contents above the critical concentration for maximum growth rate (2.2%, Pedersen and Borum 1997). Denitrification through bacterial processing may result in nitrogen displaying even higher δ^{15}N values, despite lower nitrogen loadings, and increases the loss of nitrogen from the environment (Armitage *et al.* 2006). Due to high denitrification under anoxic conditions in coastal areas of the south-eastern Pacific (Codispoti and Packard 1980), the loss of N would be intense. Furthermore, algae exposed to high concentrations of DIN can preferentially incorporate lighter N, resulting in a significant isotopic fractionation responsible for the inverse relationship between N content and δ^{15}N in *Ulva* (Thornber *et al.* 2008). As we did not analyse isotopic signatures from various sources, we cannot speculate on the relative importance of both mechanisms in this system. Nevertheless, increasing N content and lower C : N ratios in areas affected by wastewater may reflect higher N availability (Hauxwell *et al.* 1998; Rogers 1999). Thus, *Ulva* N content and C : N ratio at Des can be attributed to the observed nutrient pulses.

Community structure

There were no changes in species composition related to the distance from the outfall, in contrast to that commonly found elsewhere (e.g. López Gappa *et al.* 1990; Bishop *et al.* 2002).

In Ancón Bay, a similar suite of species was observed, but patterns of abundance differed among sites. Both mussel species were abundant at the outfall; however, during our colonisation experiment, the highest cover was reached at PC. Sewage discharge may enhance post-settlement survival through decreased starvation in areas with higher food availability (Hindell and Quinn 2000). An alternative explanation is the lateral migration of mussels, mainly *S. algosus*, which may occur faster than colonisation of denuded rock (Tokeshi and Romero 1995). Furthermore, the site with lowest colonisation, SM, resulted in a relatively low abundance of mussels. Associated with the low mussel abundance, a polychaete commonly found with mussels, *Pseudonereis* spp. (Tokeshi and Romero 1995), had the lowest density at SM. At Des and PH, the abundances of the brittle star *O. kroyeri*, another taxon closely associated with mussel beds, were also high and much more abundant than previously reported for Ancón Bay (from 1470 to 4690 ind m^{-2}, Tokeshi and Romero 1995).

Succession

This sequence of species during community development in this study on the Peruvian coast may be due to seasonal cycles or true succession. Nonetheless, environmental factors appear to be much more affected by interannual variability than by seasonal cycles. Average seasonal variation of surface water temperature is only 5–7°C, salinity only varies significantly during El Niño events when oceanic waters approach the coast (Tarazona *et al.* 2003), whereas mean air temperature ranges from 17°C in winter to 23°C in summer (Servicio Nacional de Meteorología e Hidrografía del Perú 2009). Moreover, bare space, even though it is spatially variable, was abundant and available for colonisation during the study, suggesting that over the experiment, any temporal difference in recruitment would have been evident (see Bulleri 2005).

The barnacle *J. cirratus* was abundant during the first stage of succession at all sites and was later replaced by mussels. During later stages of succession, mussels usually overgrow the early colonists, chthamaloid barnacles whose lateral walls facilitate mussel recruitment (Navarrete and Castilla 1990; Tokeshi and Romero 1995). This interaction explains the temporal variability of barnacle cover.

Exclusions at SM and PH attained higher biofilm cover than PC and Des exclusions, although differences among treatments were not detected at any site. We would expect higher biofilm cover at Des and within grazer exclusions, as nutrient availability and grazing intensity affect biofilm abundance (e.g. Hillebrand *et al.* 2000); however, a different pattern was observed, which might arise due to differences in structure type. Biofilm was the only group that presented a consistent pattern of differences between natural and artificial structures. When comparing seawalls and rocky shores made from the same material, variations at the small scale of water-motion and substratum type conditions may affect biofilm growth (Bulleri 2005). Furthermore, biofilm cover was inversely related with mussel cover over time, probably due to overgrowth by mussels. Thus, biofilm could facilitate mussel larvae settling, which in turn may shade biofilm after recruitment, as observed in September. Post-recruitment mortality of mussels would lead to the enhancement of biofilm observed in December.

The red alga *Gelidium* had no significant seasonal or spatial variation in cover, but was the only taxon affected by grazing. Interestingly, abundances in un-manipulated and control plots were higher than in exclusions. Some grazers such as *Siphonaria lessoni* are important in dispersing and redistributing propagules of filamentous opportunists and late successional algae, even increasing the number of macroalgal propagules being dispersed (Santelices and Ugarte 1987). Through digestion, *S. lessoni* may stimulate protoplast release, resulting in high survival rates of vegetative and reproductive tissues. Simultaneously, limpets may limit the growth of diatoms that compete with *Gelidium*, enhancing the effect of the latter mechanism and resulting in higher *Gelidium* abundance in the presence of grazers.

The green algae *Ulva* showed temporal variability only at SM, reaching the highest cover in September. Furthermore, *Ulva* was not affected by herbivores. Despite the often-stated generality that green ephemerals are more abundant at point-source discharges (e.g. Bishop *et al*. 2002), multivariate analysis of the mature community did not reveal differences among sites. Nevertheless, colonisation by green algae may be a result of organic enrichment in addition to reduced grazing pressure (e.g. Valiela 1995). Similar to that reported for *Patelloida latistrigata* at a larger scale along a sewage impact gradient in the Australian coast (Bishop *et al*. 2002), mobile grazers were also more abundant at the point of discharge. For instance, *Scurria* spp. and *Siphonaria* spp. were up to 6 and 24 times denser, respectively. This might indicate prevailing bottom-up effects from nutrients to grazers via macroalgae and a significant top-down feedback through increased grazing pressure preventing macroalgal overgrowth. Such dynamic links between bottom-up and top-down effects have been observed on a larger spatial scale by comparing contrasting coastlines and at a smaller scale by enrichment experiments (see Wootton *et al*. 1996; Worm and Sommer 2000).

Although coastal currents, influenced by local coastline heterogeneity, may drive differences in community patterns, at Ancón Bay, a low energy environment, there are no large headlands that could produce a major 'settlement shadow' for settling larvae (Roughgarden *et al*. 1988). Furthermore, in the south-eastern Pacific, spatial variation in recruitment of sessile invertebrates seems to be the outcome of meso-scale processes (Broitman *et al*. 2001; Lagos *et al*. 2008). Thus, it seems unlikely that coastal currents could produce significant among-site spatial variability within the bay.

In general, no top-down effects were detected during succession. Although our exclusion method may not impede the entry of some mesograzers (e.g. amphipods), the use of cumbersome devices to manipulate such animals would have probably distorted natural conditions (see Parker *et al*. 1993). Moreover, except for birds that feed on large mussels (F. Hidalgo, unpubl. data), major predators (e.g. asteroideans and carnivorous crabs) were almost absent at the mid-intertidal, and it is likely that, at upwelling sites, their effects are relatively weak at this level (see Broitman *et al*. 2001). The results of the present study also agree with other studies that pointed out the relative ineffectiveness of grazers in controlling algal development in nutrient-rich systems (e.g. Valiela *et al*. 1997; Hauxwell *et al*. 1998). Thus, the community development seems to be bottom-up controlled in the entire bay, independently of the outfall distance and nutrient

availability. It is likely that the general pattern of the intertidal assemblage is the product of processes operating at larger temporal and spatial scales, associated with oceanographic conditions and the resulting benthic-pelagic coupling (e.g. Menge *et al*. 2003). Unfortunately, there are no similar areas outside the bay free of urban influence that could be suitable as reference locations, and which would have added valuable information about the pristine state and dynamics of the community. Multiresolution studies at larger temporal and spatial scales are needed to evaluate the possible interaction of both local (sewage outfall) and regional (upwelling) effects on the observed pattern.

Our conclusions are therefore restricted to urbanised shores where most land-derived nutrient input comes from domestic sewage rather than agricultural or rainwater run-off and are under the influence of year-round upwelling. Under these conditions, we conclude that despite the localised impact of wastewater disposal, in such a nutrient-rich system environmental factors may be more important than anthropogenic-derived nutrients in shaping intertidal community structure.

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