



## Factors controlling macroalgae assemblages in a Southwest Atlantic coastal lagoon modified by an invading reef forming polychaete



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### ABSTRACT

In the Mar Chiquita coastal lagoon (37° 40'S, 57° 23'W, Argentina), the invasive reef building polychaete *Ficopomatus enigmaticus*, change the composition and abundance of macroalgae by providing habitat to some species and by excluding others. The magnitude of these effects may be regulated by consumers and nutrients availability. Here we evaluate the relative importance of consumers and nutrients in controlling macroalgae development on the reefs, and on the sediment in areas without reefs. Experiments showed that: (1) large consumers (birds, fishes and crabs) had no effect on red (*Polysiphonia subtilissima*) and green (*Cladophora* sp.) macroalgae on reefs; (2) large consumers had positive effect on green macroalgae development on the sediment of areas without reefs, probably by enhancing the availability of bivalve shells for macroalgae attachment; (3) small consumers (amphipods, hydrobiid snails and crabs) had negative effect on green macroalgae development, probably by direct consumption; (4) small consumers had positive effect on the biomass of the red macroalga *P. subtilissima*; and (5) nutrient had no effect on macroalgae. These interactions would determine the abundance and the spatial distribution of macroalgae (e.g., patchiness). Consequently, the effect of an invader ecosystem engineer in the composition of species assemblages may be mediated by other community structuring forces, which in turn may have different effects according to their interaction with the invader.

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### 1. Introduction

Ecosystem engineers are species that strongly affect the structure of the communities (Jones et al., 1994), inducing significant changes when they invade new environments (Crooks, 2002). The study of ecosystem engineer invasions is useful to understand both ecosystem engineering (see Jones et al., 2010) and invasive processes (see Crooks, 2002). The effect of an invasive ecosystem engineer can be determined by the characteristics of the species itself and its potential to change the environment (see Sousa et al., 2009). For example, invader ecosystem engineers may exclude or expand the distribution of macroalgae species due to physical (Bertness, 1984) or chemical (Hecky et al., 2004) changes they make.

Context dependent interactions are also important. In coastal environments, invertebrate grazing can regulate local patterns of diversity, abundance and distribution of macroalgae (see Heck and Valentine, 2007). These control can be by direct consumption

(e.g., Lubchenco and Gaines, 1981; Silliman and Zieman, 2001) or through physical changes mediated by their feeding activities (Nogaro et al., 2009; Schratzberger and Warwick, 1999). In addition, changes in nutrient concentration can affect the productivity of the system. For example, by modifying the herbivore pressure or the growth of certain species of macroalgae of the assemblage (see Burkepile and Hay, 2006). Thus, the balance between top-down and bottom-up factors is a recognized community regulator that may mediate the effects of ecosystem engineers (Nogaro et al., 2009), probably modulating their effects during the invasion process.

The invasive polychaete *Ficopomatus enigmaticus* (Fauvel, 1923) (Serpulidae) is distributed in brackish waters from temperate zones throughout the world (Ten Hove and Weerdenburg, 1978). *F. enigmaticus* has been reported in estuaries of the eastern South Atlantic from the mid-19th century (see Borthagaray et al., 2006; Brankevich et al., 1988; Obenat and Pezzani, 1994), being the Mar Chiquita coastal lagoon the primary area of invasion. This is a fouling organism that inhabits calcareous tubes (Bianchi and Morri, 2001). When tubes grow vertically from the substrate and cement to each other, they form reef-like structures (Bianchi and Morri, 1996). In Mar Chiquita, reefs have a mean density of 100 reefs ha<sup>-1</sup> with an average diameter of 2 m (Schwindt et al., 2001, 2004). Some areas

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of the lagoon remain unoccupied by reefs (Bazterrica et al., 2012; Bruschetti et al., 2009; Schwindt et al., 2001).

Reefs affect macroalgae assemblages within the lagoon. Reefs provide new substrate for macroalgal settlement and exclude macroalgae from the sediment of areas with reefs to areas unoccupied by reefs (Bazterrica et al., 2012). The exclusion of the macroalgae from sediment of areas with reefs is by the modification of physical and biological conditions of the surrounding benthos (i.e., higher sedimentation rates and herbivory; Bazterrica et al., 2012). On the reefs and on the sediment of areas without reefs, macroalgae are distributed in patches (Bazterrica, pers. obs.). This pattern suggests that macroalgae are controlled by a variety of factors, such as high sedimentation rates and herbivory (see Bazterrica et al., 2012).

Since macroalgae are food source and create suitable habitat for many species, the interactions described above may cascade down the food web (e.g., Buschbaum et al., 2006; Marques et al., 1997). Therefore, to know the relative importance of the factors structuring macroalgae assemblage may contribute to the understanding of the processes generated by an invader ecosystem engineer. In this paper we evaluate the relative importance of consumers and nutrients in controlling the macroalgae growing on the introduced polychaete reefs and on the sediment in areas without reefs.

## 2. Methods

### 2.1. Study site

Mar Chiquita coastal lagoon (37° 40'S, 57° 23'W, Argentina) is a UNESCO Man and the Biosphere Reserve. This body of brackish water (46 km<sup>2</sup>) is permanently connected to the sea, having a wide salinity range (2 to 35 psu; Schwindt et al., 2004) and a low amplitude tides ( $\leq 1$  m; Isla, 1997). The lagoon receives sediment and water from creeks and artificial channels (Fasano et al., 1982) that contain sustained nutrient loadings from agricultural and cattle practices from the adjacent terrestrial system (Marcovecchio et al., 2006; Orúe et al., 2011).

Experiments and observations were done in the lower intertidal of San Gabriel (Fig. 1). We identified two areas (~10 ha) located 100 m away with similar current flow. One area has high reef density, and the other area has no reefs (see Bazterrica et al., 2012). Although it is unknown why this area is devoid of reefs, we use this area as the best approximation to understand how the structuring processes would be in the absence of reefs. Areas were treated separately for sampling, experimentation and data analysis and the results were discussed comparatively.

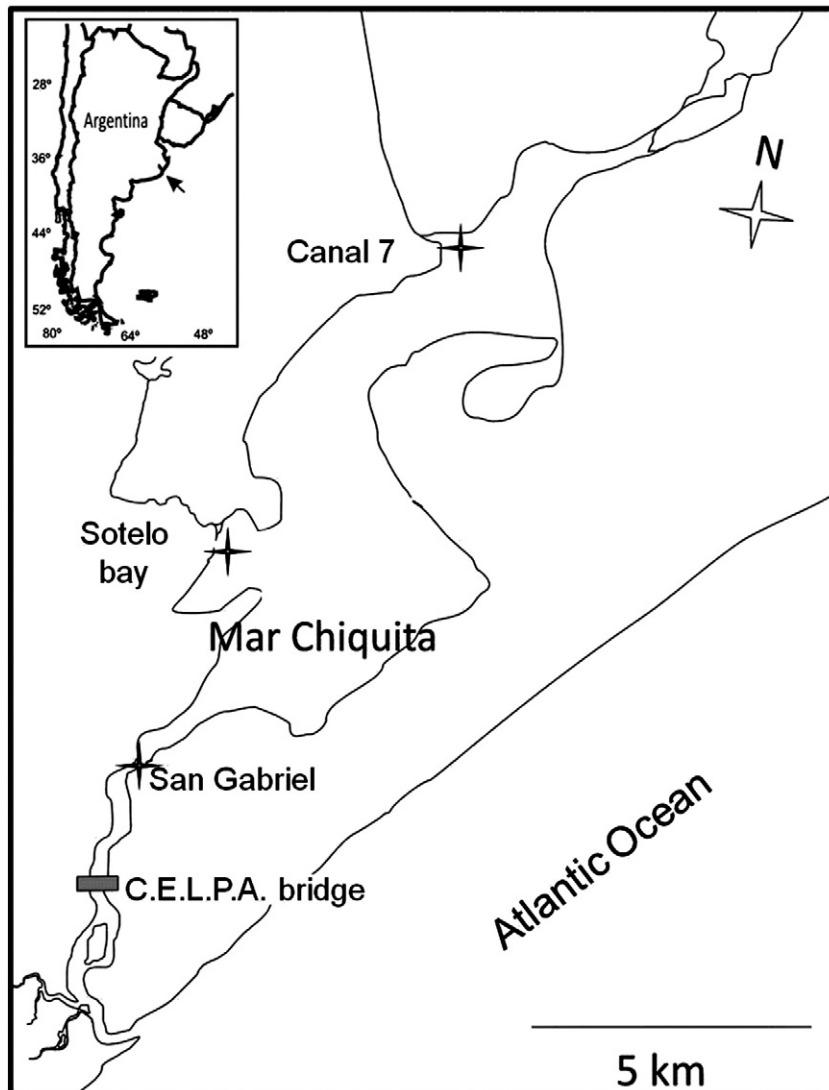


Fig. 1. Map of the Mar Chiquita coastal lagoon showing main channels and fishing sites. Reefs are present between C.E.L.P.A. and Canal 7. The sampling and experiments were done in San Gabriel.

Reef surfaces and valves on the sediment of areas without reefs are inhabited by several macrofaunal organisms, such as the amphipods *Melita palmata* (Montagu, 1804) and *Corophium insidiosum* Crawford, 1937 (Obenat et al., 2006), the snails *Heleobia* spp. (*Heleobia australis* and *Heleobia conexa*; De Francesco and Isla, 2003) and the crab *Cyrtograpsus angulatus* Dana, 1951 (Méndez Casariego et al., 2004) which could feed on macroalgae. Crabs could also affect macroalgae by burrowing and reworking the sediment (see Schratzberger and Warwick, 1999).

Birds and fishes might also affect macroalgal assemblages through physical disturbance when searching for food (e.g., birds: Freudendahl et al., 2010; birds and fishes: Thrush et al., 1991). On reef surfaces and on the sediment, birds can disturb the algae during their feeding (i.e., *Tringa melanoleuca* (Gmelin, 1789) and *Tringa flavipes* (Gmelin, 1789)) or resting activities (i.e., *Larus dominicanus* (Lichtenstein, 1823), *Larus maculipennis* (Lichtenstein, 1823), *Anas* spp., *Cygnus melanocoryphus* (Molina, 1782), *Himantopus melanurus* (Vieillot, 1817), *Vanellus chilensis* (Molina, 1782), *Milvago chimango* (Vieillot, 1816) and *Ciconia maguari* (Gmelin, 1789); see Bruschetti et al., 2009).

Fish could also remove the sediment during feeding or food searching (e.g., *Platanichthys platana* (Regan, 1917), *Jenynsia multidentata* (Jenyns, 1842), *Brevoortia aurea* (Spix and Agassiz, 1829), *Micropogonias furnieri* (Desmarest, 1823)). Particularly, *Paralichthys orbignyanus* (Jenyns, 1842) and *Oncopterus darwini* (Steindachner, 1874) (see Cousseau et al., 2001). Other species, such as *Mugil platanus* (Gunther, 1880) and *Odontesthes argentinensis* (Valenciennes, 1835) *Anas* spp. (Linnaeus, 1758) may also feed on macroalgae (Acha, 1990; Escalante, 2001). In particular, *Gobiosoma parri* (Ginsburg, 1933) (Cousseau et al., 2001) belong to a genus closely related with *F. enigmaticus* reefs that feed among tubes (Bianchi and Morri, 1996).

## 2.2. Macroalgal cover on the reef surface and on the sediment of areas without reefs

We estimated the percent cover of macroalgae and bare space on both reef surfaces and sediment from areas without reefs (December 2006). On the reef surface we used randomly allocated quadrats of 20 × 20 cm (15 quadrats were used for macroalgae and 15 for bare space). On the sediment, we used randomly allocated quadrats of 40 × 40 cm (10 quadrats were used for macroalgae and 10 for bare space). The different sizes of the quadrats ensure a representative sampling on the basis of the contrasting dimensions of each area. Percent covers were estimated by the point intercept method (100 points) from digital images of each sampling unit (see Bazterrica et al., 2007). The null hypothesis of no differences in the mean percent cover of macroalgae and bare substrate were independently compared on reef surfaces and on the sediment of areas without reefs using a Welch's *t*-test for samples with unequal variances (Zar, 1999; thereafter *tc*).

## 2.3. Effects of large consumers on macroalgae

The effect of consumers was evaluated according with consumer sizes. "Large" consumers were fishes, birds (species listed in the "Study site" section) and crabs *C. angulatus* > 1 cm carapace width (hereinafter "large crabs"; see Schwindt et al., 2001). "Small" consumers were those associated with macroalgae: amphipods (*M. palmata* and *C. insidiosum*), the snails *Heleobia* spp. and the crab *C. angulatus* < 1 cm carapace width (hereinafter "small crabs").

To evaluate the effects of large consumers we made an exclusion experiment in each area: on the reef surface and on the sediment in areas without reefs (summer 2006–2007). The experimental design had 3 treatments (N = 10 replicates per treatment): (1) total large consumers exclusion, which excluded birds, fish and large crabs; (2) bird exclusions, which allowed the entrance of fish and crabs but not birds; and (3) controls, which were no cages areas. On the

reefs, cages size was 20 × 20 × 15 cm (length × width × height). On the sediment, cage size was 60 × 60 × 50 cm. Controls on the reefs were marked areas of 20 × 20 cm. Controls on the sediment were marked areas of 60 × 60 cm. The different size of the experimental units used in each area was decided basing in previous works (see Bruschetti et al., 2009; Martinetto et al., 2011a; Schwindt et al., 2001) and according to each area dimension. All cages were constructed with a plastic mesh of the same size (1 cm) to ensure equal flow and level of exclusion at each area (see Como et al., 2006). Cages were anchored to the substrate with wood stakes on their vertices. Bird exclusions were cages opened on two opposite sides (i.e. roof and two walls) thus they worked also as manipulation controls. Experimental plots were deployed in areas without macroalgae. Algae cover was measured once, at the end of the experiment (after 30 days), with the point intercept method from digital images of the experimental plots as was described above. Percent cover of macroalgae was analyzed with one-way ANOVAs for each macroalgae species and for each area under the null hypothesis of no differences in mean cover between treatments. Here and thereafter, before ANOVAs analysis, paired Levene tests were used to evaluate homoscedasticity (Zar, 1999). When variances were heterogeneous, data were properly transformed. If homoscedasticity could not be met, ANOVAs were conducted on rank-transformed data under the null hypothesis of no differences in the mean range value (this ANOVA is robust for heterogeneous variances, Quinn and Keough, 2002). Tukey HSD test was then used to evaluate differences among treatments when necessary (Zar, 1999). When there were no differences between means, differences in the variances were discussed to consider other potential sources of variability (see Fraterrigo and Rusak, 2008).

The effect of large consumers on macroalgae may be due to indirect effects of consumers (e.g., changes in macrofauna abundance under experimental units, see Thrush et al., 1994). Therefore, we sampled the abundance of macrofauna (amphipods, hydrobiid snails and crabs) in the experimental units as a measure of potential indirect effects. Macrofauna was collected with a cylindrical core (10 cm diameter, 5 cm depth, including macroalgae when present) and sieved through a 0.5 mm mesh. Retained organisms were then identified and counted. The null hypothesis of no differences in the abundance of each species of macrofauna present (# of individuals/core) between treatments was evaluated with one-way ANOVAs (Zar, 1999).

The availability of substrate for macroalgae attachment (see Bazterrica et al., 2012) could also affect the results of the experiment in areas without reefs. Therefore, we estimated the number of valves (counting those fouled or clear) in the experimental units as a measure of substrate availability. Then, we first quantified the relationship between the number of valves and the percent cover of macroalgae with a correlation between both variables (Pearson test; Zar, 1999). Second, in order to assess potential variation in the substrate availability we test the null hypothesis of no differences in the mean abundance of valves among treatments with one-way ANOVAs (Zar, 1999). Then, we evaluated the effect of the number of valves on macroalgae cover as macroalgae percent cover divided by the number of valves for each experimental unit. The null hypothesis of no differences in mean macroalgal cover relative to the number of valves was compared among treatments with one-way ANOVAs (Zar, 1999).

## 2.4. Effects of small consumers and nutrients on macroalgae biomass

The effects of small consumers and nutrients were analyzed by measuring changes in the biomass of *P. subtilissima* and *Cladophora* sp., in two factorial designed experiments (one experiment for each species of macroalgae; summer 2009). The experiment included four treatments (8 replicates for each treatment): (1) + consumers + nutrients, (2) + consumers – nutrients, (3) – consumers + nutrients, (4) – consumers – nutrients. Algae biomass was measured before

and after an incubation period of 15 days in transparent Plexiglas cages (20 × 20 × 15 cm, length × width × height) in the field (see Martinetto et al., 2011b for cages details). To exclude consumers, two opposite walls of the cages were of plastic mesh (mesh size of 1 mm). Cages that did not exclude consumers had a plastic mesh of 15 mm to allow small consumers entering into the cages. The threads of the meshes were transparent and with a structure extremely fine (~1 mm) to minimize the shadow effect. For fertilization treatments (+ nutrients) we manipulated N and P concentrations by using perforated PVC tubes (17 cm length, 1 mm holes) fixed to the center of the cages and filled with enriched agar (1 M KNO<sub>3</sub>; 1 M NH<sub>4</sub>Cl, and 1 M KH<sub>2</sub>PO<sub>4</sub>; see Martinetto et al., 2011b). Non-fertilized treatments (– nutrients) were PVC tubes filled with non-enriched agar. Cages were attached to a floating line anchored to the substrate with wood stakes, occupying an area of 20 m<sup>2</sup> (distance between stakes: 1.5 m), and were cleaned daily to avoid fouling. The cage arrangement allowed cages to float and rotate freely during high tides at no more than 0.4 m above the sediment. Besides, cage arrangement allowed cages to rest on the sediment during low tides. Algae for incubation were collected from the reefs (*P. subtilissima*) and from the valves on the sediment (*Cladophora* sp.). Algae were weighted (blotted wet weight, precision: 0.01 g) after being carefully washed with water of the lagoon to remove macrofaunal organisms. To avoid losing algal biomass, algae mats (~10 g) were collected keeping a piece of the attached substrate (reef or valve) and were deployed inside plastic mesh bags within the cages (mesh transparent and extremely fine with 2 mm size). Biomass variation was calculated as final biomass minus initial biomass. The null hypothesis of no difference in algal biomass among treatments was compared with two-way ANOVAs (Zar, 1999).

The method of fertilization used ensured a slow and constant release of nutrients and the volumes employed (53.4 cm<sup>3</sup>) guaranteed the effective nutrient diffusion during the 15 days period (see Teichberg et al., 2008). However, we corroborated nutrient diffusion by measuring ammonium concentration inside and outside the cages at the end of the experiment in the treatments with *P. subtilissima*. For this, water samples were taken with a syringe, filtered in the field (Whatman GF/F) and freeze immediately until their analysis. The concentration of NH<sub>4</sub><sup>+</sup> was used as reference, and was measured by the blue of indophenol method (Solórzano, 1969). To assess potential differences in nutrient concentration we first compared the mean ammonium concentration among treatments using a two-way ANOVA (Zar, 1999). Then, we pooled consumer treatments within fertilized treatments and we compared the null hypothesis of no difference in ammonium concentration among fertilized, no fertilized treatments and outside the cages with a one-way ANOVA (Zar, 1999).

The mesh used in exclusion cages is effective in decreasing the number of small consumers (see Martinetto et al., 2011b). However we corroborated the effectiveness of consumer exclusions by quantifying macrofaunal abundance in all treatments. For this, we collected and counted the number of individuals for species found inside the cages at the end of the incubations. Data were compared among treatments testing the null hypothesis of no differences in the mean abundance with two-way ANOVAs for each species (Zar, 1999).

### 3. Results

#### 3.1. Macroalgal cover on the reef surfaces and on the sediment of areas without reefs

On the reefs, the cover of *P. subtilissima* (mean = 60.44, SE = 4.80) was higher than the cover of bare substrate (mean = 44.33, SE = 5.12;  $t_{c28} = 2.29$ ,  $p < 0.05$ ). In the sediment of areas without reefs, the cover of green alga (*Cladophora* sp.; mean, SE = 54.67, 7.51) was not

different from the cover of bare substrate (mean, SE = 46.70, 5.93;  $t_{c19} = 0.68$ ,  $p > 0.05$ ).

#### 3.2. Effects of large consumers on macroalgae

On the reef surfaces, large consumers did not affect the percent cover of *P. subtilissima* (one-way ANOVA, log transformed data,  $F_{2,27} = 0.03$ ,  $p > 0.05$ , Fig. 2A) and *Cladophora* sp. (one-way ANOVA, rank transformed data,  $F_{2,27} = 0.84$ ,  $p > 0.05$ , Fig. 2B) and for both macroalgae, the percent cover was less variable in total exclusions (Levene,  $p < 0.05$ ). In the sediment of areas without reefs, the percent cover of *Cladophora* sp. was lower in total exclusions (one-way ANOVA, log transformed data,  $F_{2,27} = 37.41$ ,  $p < 0.05$ ; Tukey HSD,  $p < 0.05$ ; Fig. 2C).

On the reef surfaces, there were no differences among treatments in the abundance of macrofauna (amphipods *M. palmata* and *C. insidiosum*, one-way ANOVA,  $F_{2,27} = 1.81$ ,  $p > 0.05$ ; snails *Heleobia* spp., one-way ANOVA,  $F_{2,27} = 0.22$ ,  $p > 0.05$ ; and small *C. angulatus*, one-way ANOVA, rank transformed data,  $F_{2,27} = 3.36$ ,  $p > 0.05$ ; Fig. 3). The variances were equal among treatments for the amphipods and for *Heleobia* spp. (Levene test,  $p > 0.05$ ) but variance was higher for small *C. angulatus* in total exclusions (Levene,  $p < 0.05$ ). In the sediment of areas without reefs only *Heleobia* spp. was present, with no differences in the mean abundance (one-way ANOVA,  $F_{2,27} = 3.26$ ,  $p > 0.05$ ) or variance (Levene,  $p > 0.05$ ) among treatments (Fig. 4A).

There was a positive correlation between the percent cover of macroalgae and the number of valves in the sampling units ( $r^2 = 0.27$ ,  $N = 30$ ,  $p < 0.05$ ). The number of valves was lower in total exclusions (one-way ANOVA,  $F_{2,27} = 6.51$ ,  $p < 0.05$ ; Tukey HSD,  $p < 0.05$ ; Fig. 4B). Mean macroalgal percent cover relative to the number of valves was lower in total exclusions (one-way ANOVA, ln transformed data,  $F_{2,27} = 9.28$ ,  $p < 0.05$ ; Tukey HSD,  $p < 0.05$ ; Fig. 5).

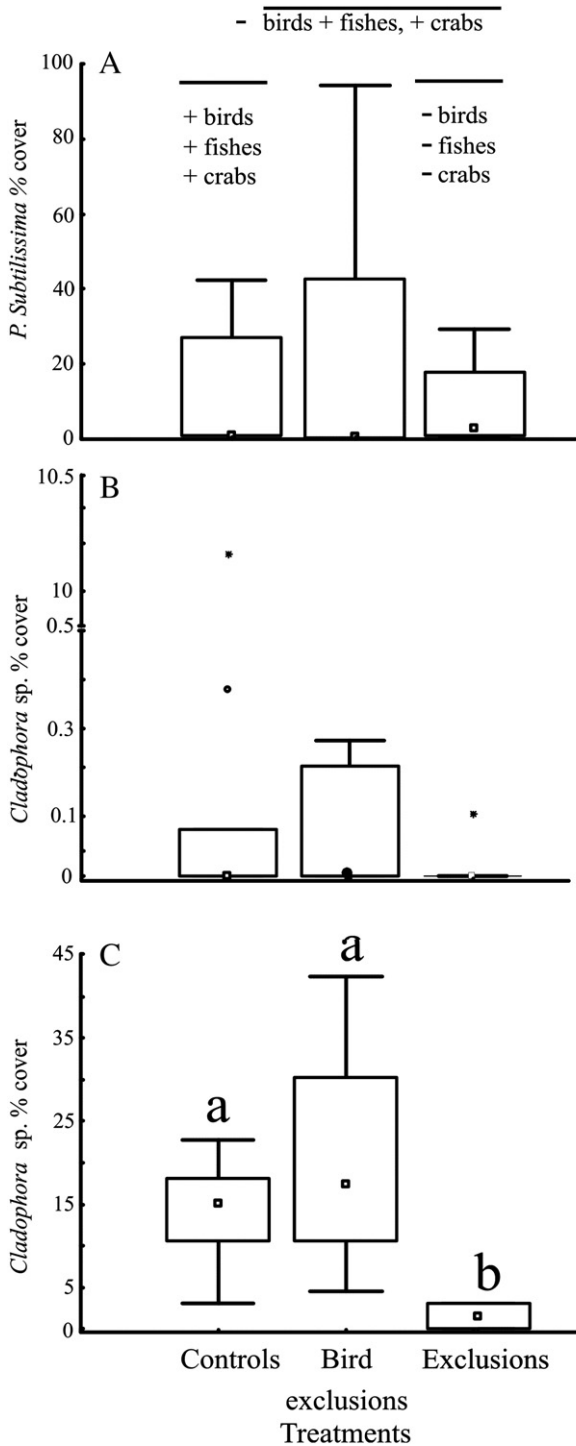
#### 3.3. Effects of small consumers and nutrients on macroalgae biomass

During the incubation period in the Plexiglas cages, the biomass of *P. subtilissima* increased in all treatments but the increase was higher in the treatments with consumers (two-way ANOVA, ln transformed data, consumers:  $F_{1,28} = 4.34$ ,  $p < 0.05$ ; nutrients:  $F_{1,28} = 0.12$ ,  $p > 0.05$ ; consumers × nutrients:  $F_{1,28} = 0.71$ ,  $p > 0.05$ ; Fig. 6A). The biomass of *Cladophora* sp. diminished at the end of incubation period, and this was higher in presence of consumers (two-way ANOVA, consumers:  $F_{1,28} = 5.48$ ,  $p < 0.05$ ; nutrients:  $F_{1,28} = 0.37$ ,  $p > 0.05$ ; consumers × nutrients:  $F_{1,28} = 3.88$ ,  $p > 0.05$ ; Fig. 6B).

In *P. subtilissima* cages, the concentration of NH<sub>4</sub><sup>+</sup> at the end of the experiment was higher in fertilized treatments irrespective of consumers presence (two-way ANOVA, rank transformed data; nutrients:  $F_{1,28} = 7.67$ ,  $p < 0.05$ ; consumers:  $F_{1,28} = 0.48$ ,  $p > 0.05$ ; nutrient × consumers:  $F_{1,28} = 0.04$ ,  $p > 0.05$ ; Tukey HSD, nutrients,  $p < 0.05$ ; Fig. 7). The concentration of NH<sub>4</sub><sup>+</sup> was higher (one-way ANOVA, log transformed data,  $F_{2,37} = 3.50$ ,  $p < 0.05$ ; Tukey HSD,  $p < 0.05$ ) in fertilized treatments (mean = 8.95 μM, SE = 1.42) than in no fertilized treatments (mean = 6.17 μM, SE = 0.65) or outside cages (mean = 5.84 μM, SE = 0.56).

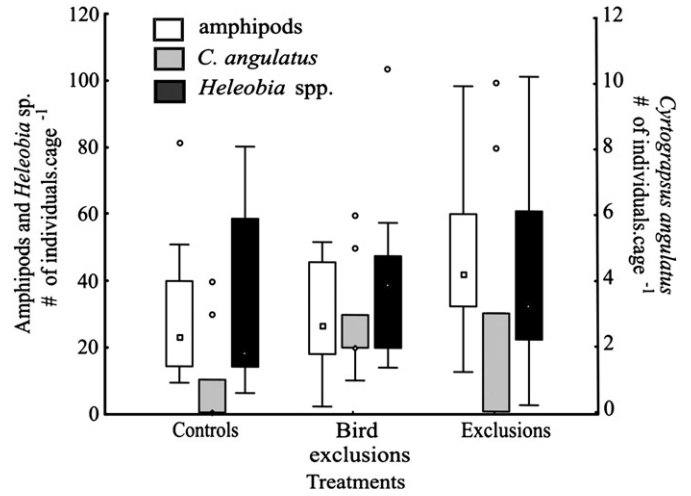
In *P. subtilissima* cages, the abundance of amphipods (two-way ANOVA, ln transformed data, consumers:  $F_{1,28} = 4.86$ ,  $p < 0.05$ ; nutrients:  $F_{1,28} = 1.31$ ,  $p > 0.05$ ; consumers × nutrients:  $F_{1,28} = 0.06$ ,  $p > 0.05$ ) and *C. angulatus* (two-way ANOVA, rank transformed data, consumers:  $F_{1,28} = 25.46$ ,  $p < 0.05$ ; nutrients:  $F_{1,28} = 0.04$ ,  $p > 0.05$ ; consumers × nutrients:  $F_{1,28} = 1.94$ ,  $p > 0.05$ ) was lower in exclusions (Tukey HSD, consumers,  $p < 0.05$ ; Fig. 8A and B). *Heleobia* spp. were more abundant in fertilized treatments (two-way ANOVA, consumers:  $F_{1,28} = 0.36$ ,  $p > 0.05$ ; nutrients:  $F_{1,28} = 6.67$ ,  $p < 0.05$ ; consumers × nutrients:  $F_{1,28} = 1.13$ ,  $p > 0.05$ ; Tukey HSD, nutrients,  $p < 0.05$ ; Fig. 8C). In *Cladophora* sp. cages, the abundance of amphipods was higher





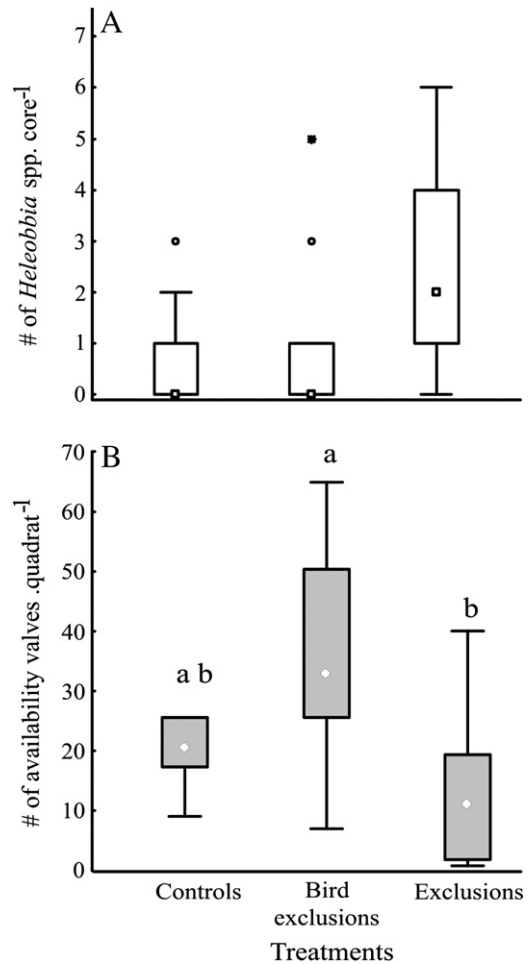
**Fig. 2.** Results of the percent cover of macroalgae in the experiments manipulating the presence/absence of large consumers. We showed the percent cover of (A) *Polysiphonia subtilissima* and (B) *Cladophora* sp. on the reef surface and the percent cover of (C) *Cladophora* sp. on the sediment from areas without reefs. Consumers absent (–) or present (+) in each treatment are shown. Hereafter: symbols within boxes are the median, box limits are 25th and 75th percentiles, bars are 5th and 95th percentiles, circles are values outside the 95% of distribution, and asterisks are extreme values. Different letters show differences for the mean among treatments (Tukey HSD test,  $p < 0.05$ ) being “a” the highest mean.

in fertilized treatments (two-way ANOVA, rank transformed data, consumers:  $F_{1;28} = 5.45$ ,  $p > 0.05$ ; nutrients:  $F_{1;28} = 2.89$ ,  $p < 0.05$ ; consumers x nutrients:  $F_{1;28} = 0.22$ ,  $p > 0.05$ ; Fig. 8D). The abundance of *C. angulatus* was lower in exclusion treatments (two-way ANOVA,

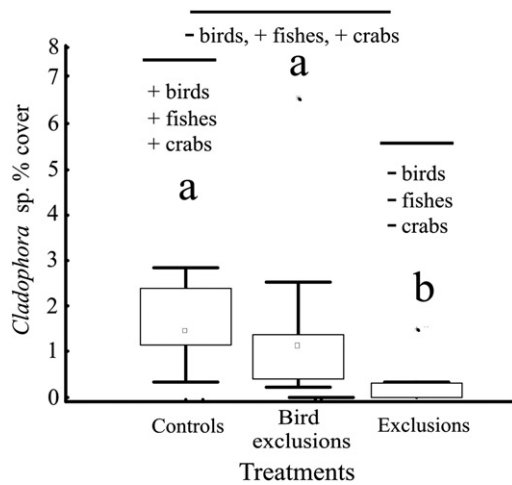


**Fig. 3.** Number of amphipods (left axis), *Heleobia* spp. (left axis) and *Cyrtograpsus angulatus* (right axis) among treatments of the experiment manipulating the presence/absence of large consumers on the reef surface.

rank transformed data, consumers:  $F_{1;28} = 68.71$ ,  $p < 0.05$ ; nutrients:  $F_{1;28} = 0.07$ ,  $p > 0.05$ ; consumers x nutrients:  $F_{1;28} = 0.07$ ,  $p > 0.05$ ; Tukey HSD, consumers,  $p < 0.05$ ; Fig. 8E). The abundance of *Heleobia* spp. was no different among treatments (two-way ANOVA,

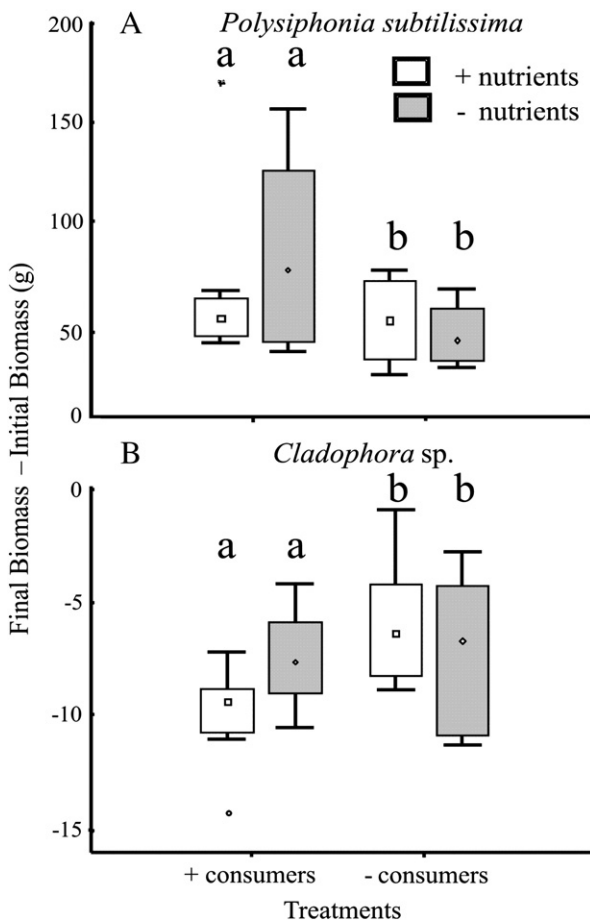


**Fig. 4.** (A) Number of *Heleobia* spp. and (B) number of valves among treatments of the experiment manipulating the presence/absence of large consumers in the sediment of areas without reefs. Different letters show differences for the mean among treatments (Tukey HSD test,  $p < 0.05$ ) being “a” the highest mean.

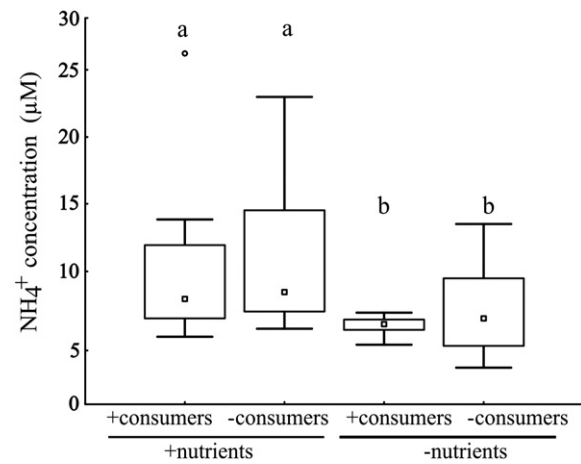


**Fig. 5.** *Cladophora* sp. percent cover/number of valves in each experimental unit on the sediment or areas without reefs. Consumers absent (–) or present (+) in each treatment are showed. Different letters show differences for the mean among treatments (Tukey HSD test,  $p < 0.05$ ) being “a” the highest mean.

consumers:  $F_{1,28} = 0.33$ ,  $p > 0.05$ ; nutrients:  $F_{1,28} = 2.28$ ,  $p > 0.05$ ; consumers  $\times$  nutrients:  $F_{1,28} = 1.28$ ,  $p > 0.05$ ; Fig. 8F) but the abundance was more variable in the “–consumers + nutrients” treatment (Levene,  $p < 0.05$ ).



**Fig. 6.** Biomass variation of (A) *Polysiphonia subtilissima* and (B) *Cladophora* sp. in the experiment manipulating the presence/absence of small consumers and nutrient addition. Positive values indicate biomass increment; negative values indicate biomass decreasing. Different letters show differences for the mean between consumer treatments (Tukey HSD test,  $p < 0.05$ ) being “a” the highest mean.



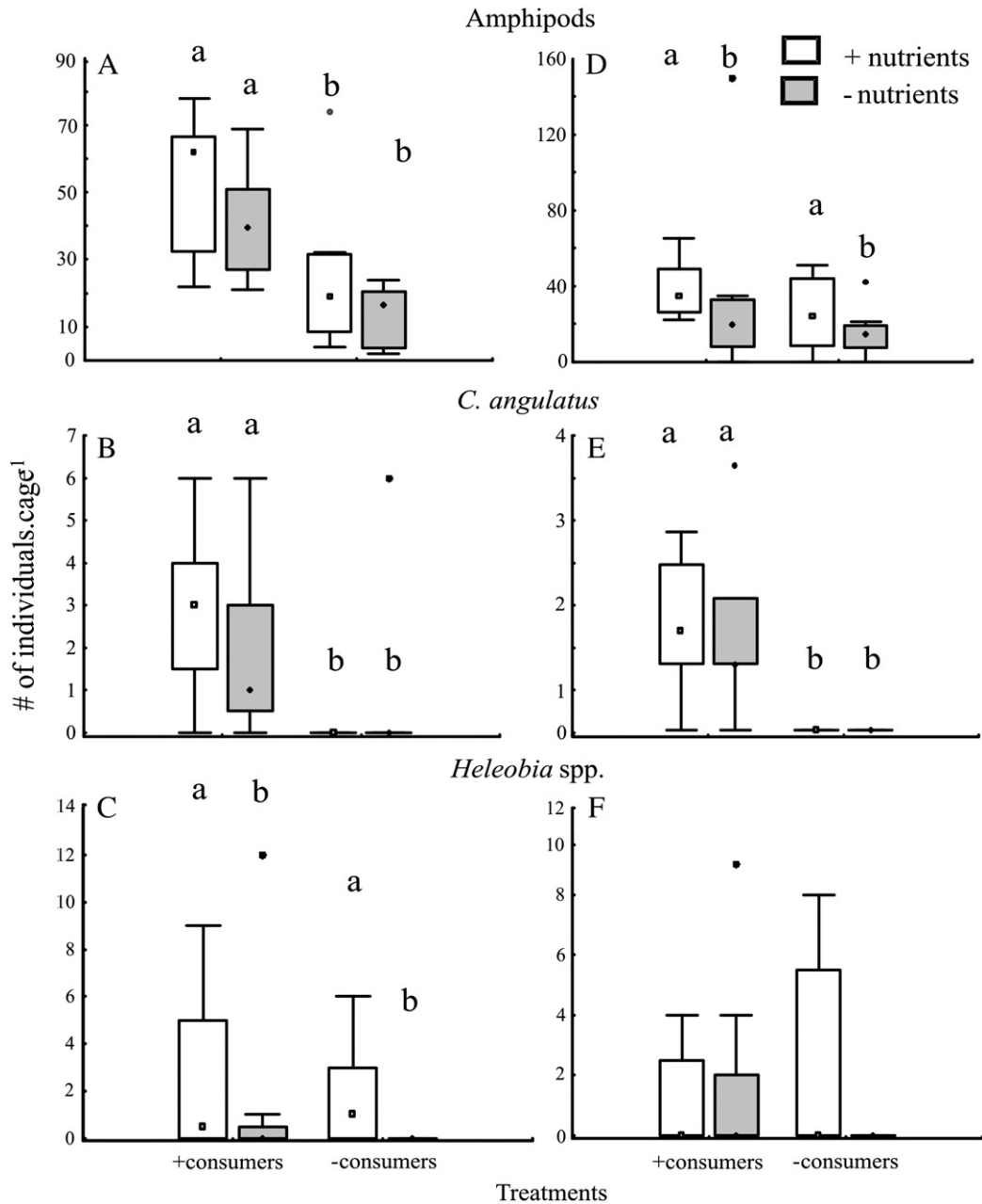
**Fig. 7.** Mean ammonium concentration within nutrient and consumer treatments at the end of the incubation experiment of *Polysiphonia subtilissima*. Different letters show differences for the mean between fertilization treatments (Tukey HSD test,  $p < 0.05$ ) being “a” the highest mean.

#### 4. Discussion

Our experiments showed that large consumers increased macroalgae biomass (*Cladophora* sp.) on the sediment of areas without reefs, but had no effect on macroalgae biomass on reef surfaces (*P. subtilissima* and *Cladophora* sp.). Small consumers (mainly amphipods and small crabs) positively affected *P. subtilissima* and negatively affected *Cladophora* sp. (mainly small crabs). Nutrient addition had not effects on macroalgal biomass.

Small consumers (amphipods, hydrobid snails and small crabs) forage, or search for food, within the macroalgae. Large consumers (birds, fishes, and large crabs) do it from outside the thallus (Hay et al., 1989). In both cases, due to consumption or disturbance, feeding activity may affect macroalgal development (e.g., Bruno and O'Connor, 2005; Lodge et al., 1994). In our work, the development of macroalgae on the reef surface was not affected by large consumers. This pattern could be explained by a low abundance of consumers or by a low consumption efficiency (see Menge, 1992). For example, the abundance of the fish *G. parri*, a species that feed among reef tubes (Bianchi and Morri, 1996), is scarce in the lagoon (Bazterrica, pers. obs). But, there could be other source of variability affecting macroalgae since the increment in macroalgal biomass was highly variable when large consumers were present. For example, factors such as top-down control of grazers by large consumers (e.g., Hamilton et al., 2006; Korpinen et al., 2007) or uncontrolled variations in macrofaunal abundance (e.g., migration from and toward cages, see Thrush et al., 1994) may be affecting macroalgae development. In our experiment, the abundance of small crabs was more homogeneous in treatments without cages. Thus, cages and bird exclusions may represent a source of disturbance in the movement of crabs because cages may act as traps or can be selected for shade and shelter. However, despite these sources of variability, macroalgae development does not suffer biological disturbances on reef surfaces due to foraging activities of birds, fish, and large crabs.

In the sediment of areas without reefs, the exclusion of large consumers favored the development of patches without algae since green macroalga cover was higher in bird exclusions and controls. In the bird exclusions and controls, large crabs (Bazterrica, pers. obs.) and valves were also more abundant. This pattern suggests a positive and indirect effect of large crabs on macroalgae. This effect could be mediated by the availability of substrate (i.e., mollusk shells) given that crabs increase the availability of superficial valves by sediment removal (see Schwandt et al., 2001). Moreover, shorebirds and fish could also disturb the sediment during feeding (e.g., birds;



**Fig. 8.** Number of individuals among treatments of the experiments manipulating the presence/absence of small consumers and nutrients. We show the number of (A) amphipods, (B) *Cyrtograpsus angulatus* and (C) *Heleobia* spp. within cages with *Polysiphonia subtilissima*, and the number of (D) amphipods, (E) *C. angulatus* and (F) *Heleobia* spp. within cages with *Cladophora* sp. Different letters show differences for the mean between treatments (Tukey HSD test,  $p < 0.05$ ) being "a" the highest mean.

Freudentahl et al., 2010; fishes: Thrush et al., 1991), including birds (see Bruschetti et al., 2009) and fishes (*M. platanus*, *O. argentinensis*, *P. orbignyana* and *O. darwinii*; see Cousseau et al., 2001) present in the coastal lagoon. Additionally, our experiment showed no effect of large consumers on the abundance of macrofauna, at least for the gastropods *Heleobia* spp. since it was the only species found. Therefore, large consumers affect macroalgae development probably by exposing the valves for attachment.

We did not observe an increment in biomass of *P. subtilissima* and *Cladophora* sp. when exposing them to higher concentrations of P and N, even when fertilization was effective since nutrient concentration was higher in the fertilized cages. This may be a consequence of several scenarios. For example, because the nutritional status of macroalgae (physiological responses determined by the reserve of nutrients from algal thalli, Pedersen and Borum, 1996, 1997) or

because the threshold nutrient concentration was not attained (see Lapointe, 1997). Besides, the interactions with small consumers may also be important. For example, during the incubation of *Cladophora* sp. we observed a negative effect of small consumers together with an increase in the abundance of the gastropods *Heleobia* spp. and amphipods with nutrient addition. Thus, a potential positive effect of nutrients may be counteracted by selective consumption of fertilized macroalgae (see Worm et al., 2000). For *P. subtilissima*, we observed that small consumers slightly increased *P. subtilissima* biomass. This increment in biomass was more variable without nutrients and less variable with nutrients and without small consumers. Considering that variability may provide additional information on the existence of important interactions that should be taken into account in data interpretation (Fraterrigo and Rusak, 2008), our results suggest that the joint effect of small consumers and nutrients

could affect *P. subtilissima* biomass. For example, herbivores may stimulate algae growth by removing the epiphytes (e.g., Kamermans et al., 2002; Russo, 1988) while epiphytes would be positive affected by nutrients (Vogt and Schramm, 1991).

We have underlined some mechanisms that control the distribution and abundance of macroalgae, which are primarily affected by the invasion of *F. enigmaticus* reefs. We found that small consumers (primarily amphipods and crabs) enhance the development of *P. subtilissima* (Fig. 9A), probably mediating the positive effect of reefs on red macroalgae (see Bazterrica et al., 2012). In the sediment of areas without reefs, small consumers (primarily crabs) can negatively affect green macroalgae through consumption (Fig. 9B). Large consumers no affect macroalgae on reefs (Fig. 9C) and may benefit macroalgae in the sediment by exposing the valves where they attach (Fig. 9D). However, small consumers could also have negative effects on reef surfaces that contribute to the formation of bare patches. In our experiments with small consumers we evaluated the effects on adult plants, but other developmental stages, such as recruitment (e.g., Vadas et al., 1992) could be affected (Diaz-Pulido and McCook, 2003). For example, species such as the snails *Heleobia* spp. can consume or bulldoze algae sporelings while foraging on the substrate (see Canepuccia et al., 2007). In turn, other mechanisms may be considered in the generation of bare patches (e.g., detaching of the extremes of the tubes holding mats of *P. subtilissima*; Bazterrica, pers. obs.). Nevertheless, the proportion of bare areas is lower than the proportion of areas covered by macroalgae, indicating that the reef surface is a suitable habitat for the development of *P. subtilissima*.

The mechanisms underlined would determine the abundance of macroalgae, the state of algal mats (e.g., degree of epibiosis, mechanic split of the thallus), and also the environmental heterogeneity (e.g., algal covered or bare patches). Similarly, consumers contribute with the exclusion of macroalgae from sediment of areas occupied with reefs (Bazterrica et al., 2012). Overall, other reef-forming species could provide the same results found in this work. However, our comparative study shows that the effect of the invader ecosystem engineer on the composition of native assemblages could be mediated by other structuring forces that may change their direction in the absence of the invader (e.g., macrofauna negatively affect macroalgae on the sediment, while have slightly positive effects on the reefs). Our findings emphasize the importance of including varying structuring forces in studies on invasive processes.

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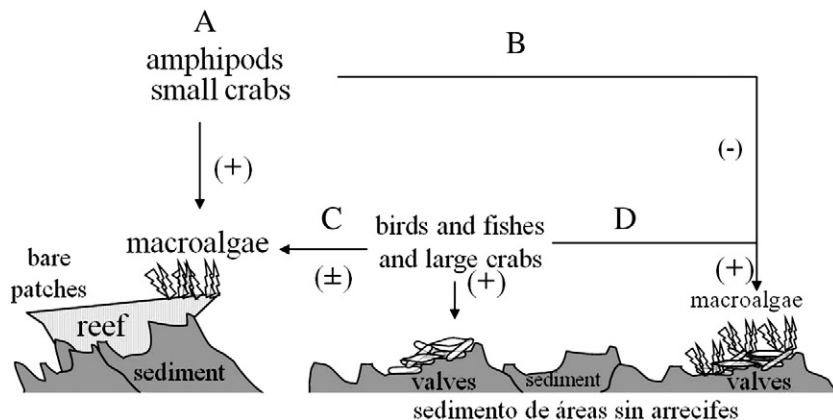


Fig. 9. Scheme of the effects of consumers on the macroalgae on the reefs and in the sediment of areas without reefs. Arrows indicate the direction of the effect; +, – and ± indicate positive, negative and neutral effects, respectively, on macroalgae in A, B, C and in valves and macroalgae in D.



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