



Effects of macroalgae on the recruitment, growth, and body condition of an invasive reef forming polychaete in a south-western Atlantic coastal lagoon



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ABSTRACT

Species interactions could mediate species invasive processes. In Mar Chiquita coastal lagoon (37° 40'S, 57° 23'W, Argentine), the invasive reef building polychaete *Ficopomatus enigmaticus* (Fauvel 1923) enhances the biomass of the red alga *Polysiphonia subtilissima* Montagne 1840 on reef surfaces, and excludes green macroalgae (mainly *Cladophora* sp. Kützing, 1843) from sediment between reefs. In turn, macroalgae could have several community structuring effects (e.g., as food or by competing for space). Therefore, macroalgae may affect *F. enigmaticus*. To evaluate this hypothesis we studied (1) the interaction between macroalgae and *F. enigmaticus* during the colonization of new substrates and (2) the effects of macroalgae on the recruitment, growth, and body condition of *F. enigmaticus*. Field sampling and experiments suggested a lack of competition on new substrates. However, there was a positive effect of macroalgae on *F. enigmaticus* during the warm season, since its recruitment, tube length, and body condition were higher in areas with macroalgae on reef surfaces. Considering that previous studies showed that reefs positively affect macroalgae, our results suggest that there is a positive feedback on *F. enigmaticus* created by macroalgae on established reefs and during the reefs' growing season. This interaction may contribute to the maintenance and growth of established reefs.

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1. Introduction

Biological invasions are an important research topic because of the impact they can have on the conservation of natural ecosystems (e.g., Troost, 2010; Vitousek et al., 1996) through changes of ecosystem states (Sakai et al., 2001). When the invader is an ecosystem engineer, changes in the habitat structure are an important pathway of community modification (Crooks, 2002, 2009). Ecosystem engineers modify the physical environment through their own structures and modulate the availability of biotic and abiotic resources for other species (e.g., shelter from predators or physical stress, Jones et al., 1994), and can indirectly affect their own performance (i.e., ecosystem engineering feedback, Jones et al., 2010).

Concurrently, species interactions may mediate invasive processes. For example, consumption could promote a patchy distribution, or the exclusion, of species primarily affected by an invasive species (see Bazterrica et al., 2012, 2013). In particular, invading ecosystem engineers may be affected by ecosystem engineering feedback (see Jones and Gutiérrez, 2007). For example, feedback could enhance the ecosystem

engineer nutritional state or decrease their growth, abundance and distribution at a landscape scale (see Bouma et al., 2009). These interactions may be important factors affecting invasive processes.

The invasive reef forming polychaete, *Ficopomatus enigmaticus* (Fauvel 1923), is distributed worldwide in estuarine environments (e.g., North America: Cohen and Carlton, 1995; UK: Thorp, 1994; Italy: Bianchi and Morri, 1996; Spain: Fornós et al., 1997; Africa: Davies et al., 1989; Asia and Oceania: Read and Gordon, 1991). In estuaries of the Southwestern Atlantic, *F. enigmaticus* was reported in the mid-19th century (Borthagaray et al., 2006; Brankevich et al., 1988; Obenat and Pezzani, 1994). In the Mar Chiquita coastal lagoon (37° 40'S, 57° 23'W, Argentina; A UNESCO Man and the Biosphere Reserve), reefs are large, reaching up to 0.5 m in height and 7 m in diameter (Schwindt et al., 2001). There, reefs occupy up to 86% of the coastal lagoon (Schwindt et al., 2001) with densities of about 89 reefs·ha⁻¹ (Schwindt et al., 2004b), but there are several areas without reefs (see Bazterrica et al., 2012).

In the Mar Chiquita coastal lagoon, biomass of the red macroalga *Polysiphonia subtilissima* Montagne 1840 is highest on reef surfaces, but reefs exclude macroalgae (mainly the green macroalga *Cladophora* sp.) from surrounding sediments (Bazterrica et al., 2012). The effects of *F. enigmaticus* on macroalgae could have consequences for other trophic levels, since macroalgae have several mechanisms to structure communities: as autogenic ecosystem engineers (Stewart and Carpenter, 2003; Wallentinus and Nyberg, 2007), by bottom-up control

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as food (Alfaro et al., 2006; Bruno and O'Connor, 2005), by assimilation/dissimilation processes (Jones and Gutiérrez, 2007) and also by other interactions such as competition (Lubchenco, 1982; McCook et al., 2001).

Ficopomatus . enigmaticus and macroalgae might compete for new substrates for recruitment since both recruit on the same substrate (i.e., bivalves and snail valves (Bazterrica et al., 2012; Schwindt and Iribarne, 2000)). Additionally, macroalgae could enhance (or reduce) the recruitment and development of *F. enigmaticus* in substrates occupied by macroalgae (valves and reef surfaces; Bazterrica et al., 2012; Obenat and Pezzani, 1994; Schwindt and Iribarne, 2000). For example, macroalgae may positively affect *F. enigmaticus* recruitment by enhancing the refuge, modifying physical conditions (e.g., Daleo et al., 2006; Stewart and Carpenter, 2003), or enhancing food concentration (see Rabaut et al., 2007; Wahl, 1989). On the other hand, macroalgae could negatively affect *F. enigmaticus*, for example, by interfering with their filtering performance (see Dittman and Robles, 1991).

The morphology of the tubes of *F. enigmaticus* is variable according to environmental or biological conditions (e.g., wall thickness of tubes depends on the distance between individuals, Nishi and Nishihira, 1997; tube growth rates are low during gametogenesis, Bianchi and Morri, 1996). The biomass of the worms also can increase under appropriate temperature and nutrient conditions (Schwindt et al., 2004a). Therefore, several parameters of *F. enigmaticus* morphology may change according to macroalgae presence, including the shape of the tubes (length) and the body condition of the worms inside the tubes (weight · height⁻¹).

Reef spread is thought to be mainly controlled by environmental variables (e.g., salinity, nutrients, and current speed; Bianchi and Morri, 2001; Schwindt et al., 2004b) and the availability of nuclei (i.e., hard substrate for settlement of the polychaete that may generate a new reef; see Schwindt et al., 2004a). Less evidence exists about biotic controlling factors (e.g., predation, competition; see Bianchi and Morri, 2001; Schwindt et al., 2004b). A possible biotic factor is the effect of macroalgae on the density and growth of the reefs of *F. enigmaticus* by affecting their colonization of new substrates or the addition of new individuals to reef surfaces. But also macroalgae could affect the tube length or worm biomass, having cascading effects on processes such as fertility and gamete production (see Charles et al., 2003; Dittman and Robles, 1991). Therefore, our objective is to investigate the effect of macroalgae on *F. enigmaticus* in the colonization of new bare substrate, in their recruitment on areas previously occupied with macroalgae, and in their growth and body condition.

2. Methods

2.1. Study site

We worked in the Mar Chiquita coastal lagoon, a body of brackish water (46 km²) permanently connected to the sea (Isla, 1997). It has a wide salinity range (2 to 35; Schwindt et al., 2004a, 2004b) with low amplitude tides (≤ 1 m) that decrease towards the main body of the lagoon (Isla, 1997). *Ficopomatus F. enigmaticus* reefs are found everywhere from C.E.L.P.A. bridge to Channel 7 (Fig. 1), with the highest abundance in the center (Schwindt et al., 2004b). To accomplish our objective, we performed surveys and field experiments in the low intertidal (a site located approximately 6 km inland from the lagoon inlet; San Gabriel; Fig. 1).

2.2. Relationship between *Ficopomatus F. enigmaticus* and macroalgae recruitment on bare substrates

We described *F. enigmaticus* and macroalgae recruitment on new hard substrate, following the recruitment of both groups of organisms on initially bare valves of the snail *Adelomelon brasiliana* (Lamarck 1811). We used valves of *A. brasiliana* because they are the main “nuclei”

selected by *F. enigmaticus* (Schwindt and Iribarne, 2000). Available valves were collected from C.E.L.P.A. to the inlet of the lagoon (n = 30; Fig. 1). The size range of the valves was 8 to 14 cm between the apex and the siphonal channel (see Fig. 2A). Valves were brushed and placed on sediment tied individually with nylon string (1 m long) to numbered wooden stakes (2 m high). The nylon string was glued to the apex of the valves with a small pellet of putty epoxy. Wooden stakes were buried (1 m depth) in a line (~6 m) parallel to the coast (mean depth during low tide = 0.93 m, SE = 0.10; measured in January 2008 over 11 days). The experiment started in October 2007 and data were collected in December 2007, and January, February, April, June, August and October 2008. The abundances of *F. enigmaticus* (number of recruits · valve⁻¹) and macroalgae (percent cover · valve⁻¹) were registered visually, considering two sides of the valves: the internal and external sides (see Fig. 2A and B). The null hypothesis of no differences in the abundance of *F. enigmaticus* and macroalgae was evaluated with repeated measures ANOVA that included month and valve side (internal and external) as factors (Zar, 1999; n = 10 due to the loss of some experimental units).

During the experiment we observed the recruitment of the barnacle *Balanus improvisus* and the presence of the crab *Cyrtograpsus angulatus*. Both species may affect *F. enigmaticus* and macroalgae. Barnacles may compete for the substrata (e.g., Anderson, 1999) and crabs may consume and/or disturb *F. enigmaticus* (e.g., Schwindt and Iribarne, 2000). To evaluate potential relationships between the abundance of *B. improvisus* (# of individuals · valve⁻¹) and macroalgae percent cover on the external side of the valve; and between the abundance of *C. angulatus* (# of individuals · valve⁻¹) and the number of *F. enigmaticus* recruits inside the valves Spearman correlations (Zar, 1999) were performed.

2.3. Macroalgae effects on recruitment of *Ficopomatus F. enigmaticus*

2.3.1. Description of the recruitment of *Ficopomatus F. enigmaticus* on hard substrates, with and without macroalgae

The recruitment of *F. enigmaticus* in the presence or absence of macroalgae was quantified on the hard substrates available in the sediment on the bottom of the lagoon (“nuclei”) in order to assess a potential relationship between both organisms in the use of the nuclei. For that, we sampled the abundance of *F. enigmaticus* and macroalgae on nuclei of the following categories, collected randomly from the lagoon (January 2008): valves of *Mactra isabelleana* d'Orbigny 1846, valves of *Tagelus plebeius* Lingtfoot 1785, valves of *A. brasiliana* and “others” (i.e., plastics, bottles, etc; see Schwindt and Iribarne, 2000). We determined that a greater number of recruits of *F. enigmaticus* in the presence of macroalgae would suggest a positive interaction. In contrast, a lower number of recruits of *F. enigmaticus* with macroalgae would suggest a negative effect. Finally, if the abundance of recruits of *F. enigmaticus* did not vary, we would conclude no potential effect of macroalgae. The null hypothesis of no differences in the abundance of *F. enigmaticus* and macroalgae in each category of nucleus was tested with a one-way ANOVA (Zar, 1999).

We observed variations in the position of the valves on the sediment, and in the position of macroalgae and *F. enigmaticus* on valves. In order to describe these patterns, we recorded the resting position of the valves and the side of the valves occupied by *F. enigmaticus* and macroalgae. Resting positions were “convex” (i.e., with the internal side, or mantle, of the valve on the sediment; Fig. 2C and D), “concave” (i.e., with the external side of the valve on the sediment; Fig. 2C and D) and “semi-buried” (i.e., semi-buried or upright valves; only considered for bivalve valves); for *A. brasiliana*, the position “convex” corresponded with valves lying with the inside on the sediment, and “concave”, with the valve lying with the outside on the sediment (Fig. 2A). The side of the valve occupied by *F. enigmaticus* and macroalgae was classified as internal or external (see Fig. 2A, C and D). Thus, combining both the resting position and the side of the valve occupied, we considered the following “positions” for *F. enigmaticus* and macroalgae: (1) convex-

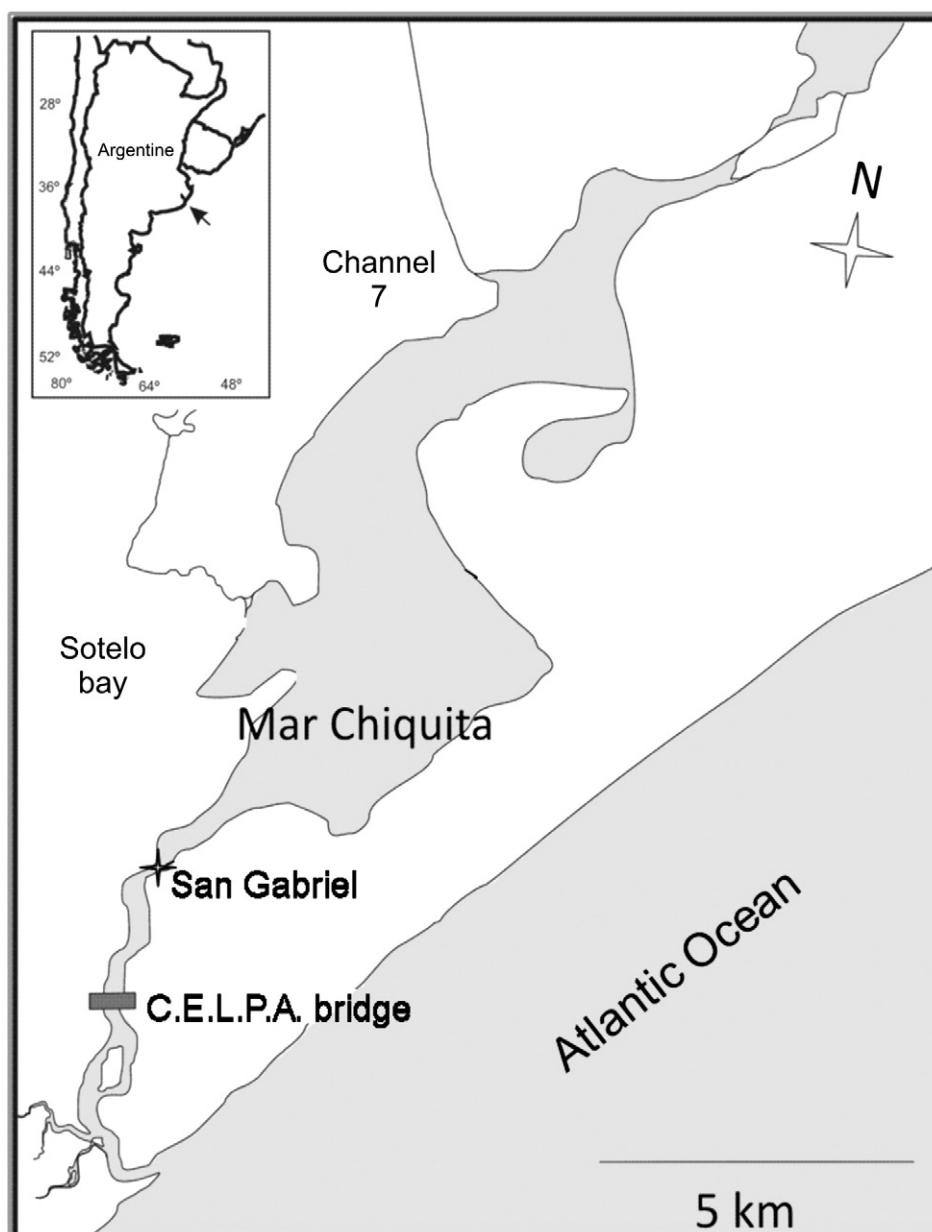


Fig. 1. The Mar Chiquita coastal lagoon.

external (valves in convex position, with organisms on the side of the valve facing the sun); (2) convex-internal (valves in convex position, with organisms on the side of the valve facing the sediment); (3) concave-external (valves in concave position, with organisms on the side of the valve facing the sun); and (4) concave-internal (valves in concave position, with organisms on the side of the valve facing the sediment). The null hypothesis of no differences in the abundance of *F. enigmaticus* and macroalgae among positions for each type of nucleus was tested with two-way ANOVAs (position \times valve side; Zar, 1999). The results for the semi-buried position are shown graphically since they were only considered for *M. isabelleana* and *T. plebeius*.

2.3.2. Description of the recruitment of *Ficopomatus F. enigmaticus* on reef surfaces with and without macroalgae

The recruitment of *F. enigmaticus* on reef surfaces with and without macroalgae was quantified. Given the difficulty of recognizing new recruits inside the tube matrix of a reef, we transplanted reefs without

polychaetes inside their tubes. Thus, recruits were identified by counting tubes with living organisms under a binocular microscope ($3\times$ and $40\times$). Reefs without polychaetes were made extracting cylindrical reef pieces with a PVC tube (5 cm in diameter; 15 cm in height) from the center of areas without macroalgae of randomly chosen reef surfaces. Pieces of reefs were kept outside the water for several days. Then, at the beginning of the recruitment season (October 2009; Obenat and Pezzani, 1994), the pieces of reefs were planted in the center of 20 areas with and without macroalgae that were randomly chosen. We monitored the presence of recruits in the experimental units weekly by randomly sampling 15% of the experimental units ($n = 3$ per treatment). The experimental units sampled were replaced with reef pieces that had been collected using the methods above. When recruits were observed (first half of January 2010), reef pieces were collected to count recruits. The null hypothesis of no differences in the number of recruits between areas with and without macroalgae was tested with a t test for heterogeneous variances (“tc test”; Zar, 1999).

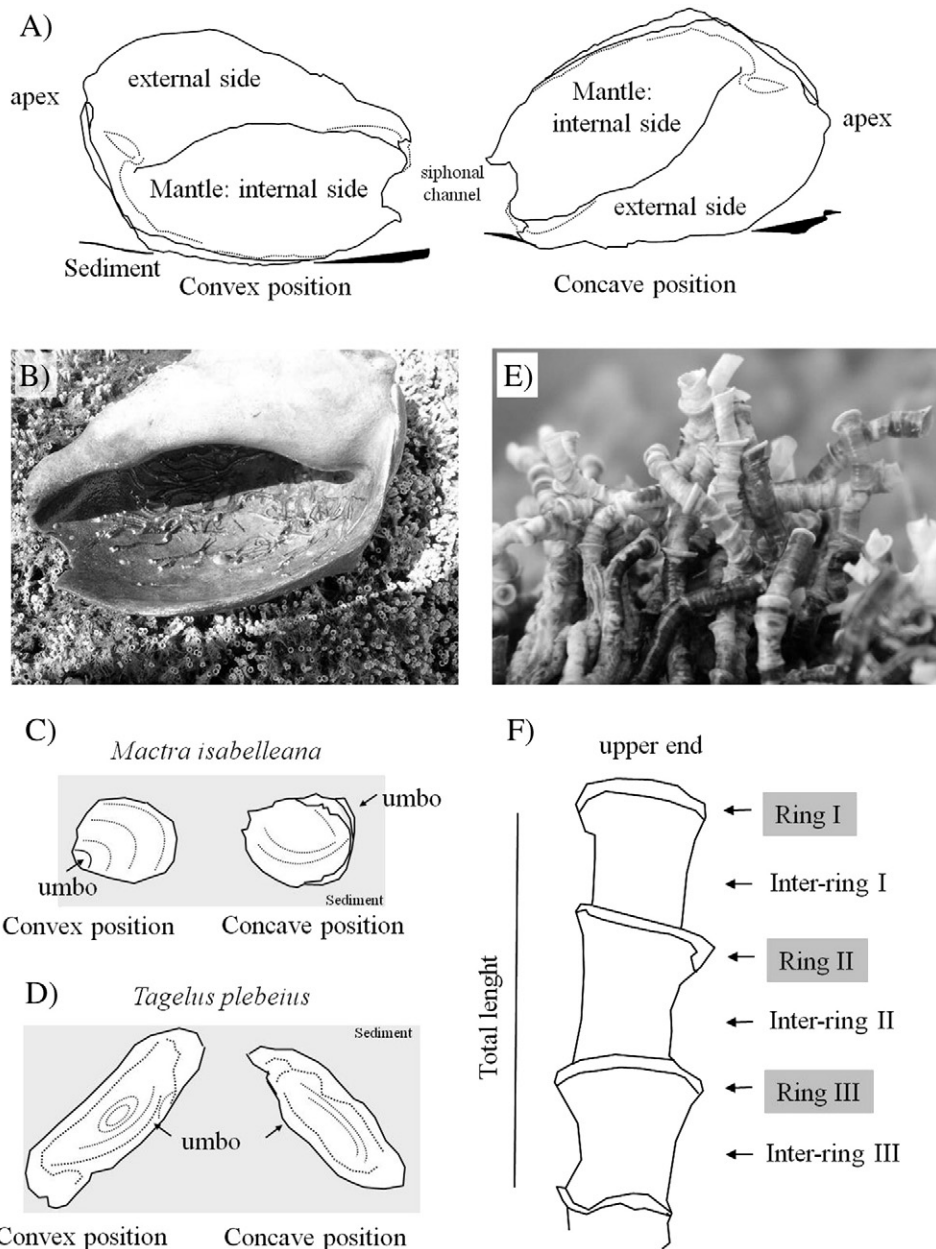


Fig. 2. (A) Scheme of the valves of *Adelomelon brasiliana* showing their internal and external sides and the position on the sediment; (B) photo showing the recruitment of *F. enigmaticus* on *A. brasiliana* valves; scheme of valves of (C) *Mactra isabelleana* and (D) *Tagelus plebeius*, showing their internal and external sides and the position on the sediment; (E) photo showing the aggregates of *F. enigmaticus* tubes, in which can be observed, for individual tubes, the rings and the inter-rings. (F) Scheme of a tube of *F. enigmaticus* showing the rings, inter-rings and the total length (modified from Schwindt et al., 2004b). The average lengths (cm) of the longest axis of the bivalve valves used were the following: *M. isabelleana*: 3.85, SE = 0.6; *T. plebeius*: 5.64, SE = 0.7.

2.4. Growth and body condition of *Ficopomatus F. enigmaticus* with and without macroalgae

2.4.1. Description of the growth and body condition of *Ficopomatus F. enigmaticus* with and without macroalgae

To describe differences in the growth of *F. enigmaticus*, we compared the tube length and an estimate of the body condition of individuals of *F. enigmaticus* between areas with and without macroalgae. For that, we randomly collected polychaete tubes (see Fig. 2E) from areas with and without macroalgae (1 tube per area for each parameter) of randomly selected reefs ($n = 30$ reefs; mean diameter = 4.62 m, SE = 3.83) at the same distance from the coast (mean depth during low tide = 0.57 m, SE = 0.02; measured in December 2008; $n = 20$ days). Macroalgae proliferation on reef surfaces

(mainly *P. subtilissima*, Bazterrica et al., 2012) and the growth of *F. enigmaticus* tubes (Schwindt et al., 2004a) is higher in summer. Thus, we sampled in December 2008 (“warm season”, n without macroalgae = 42; n with macroalgae = 46) and July 2009 (“cold season”, n without macroalgae = 40; n with macroalgae = 26). The number of tubes measured (precision 0.01 mm) within treatments was different because: the identification of rings II and III is not always evident for tubes with macroalgae; the macroalgae patches are scarce in cold seasons; and during one period of growth more than one peristomial ring could be formed (~9 weeks, Straughan, 1972). We measured the length of the tube between successive peristomial rings (*sensu* Straughan, 1972): inter-rings I, II and III (from Schwindt et al., 2004b; see Fig. 2F; precision 0.01 mm). Measurements were done on digital photographs, using the software ImageJ 1.34S (<http://www.imagej.com>). To minimize errors

due to these particularities, we defined a fourth measure of length for the analysis: the total length (inter-ring I + inter-ring II + inter-ring III; Fig. 2D). To estimate body condition, the worms inside the tubes (n with and without macroalgae = 10 of each) were measured on digital photographs as was described, and dried (60 °C; days: 3) before weighing. We calculated the body condition as $\text{dry weight} \cdot \text{length}^{-1}$ (precision 0.0001 g; 0.01 mm) of the worms.

Additionally, because tube length is inversely proportional to the degree of aggregation (Straughan, 1972), we estimate the degree of aggregation between areas with and without macroalgae, by comparing the number of tubes observed with the naked eye and with 1 cm of water over the surface. We considered that a small number of visible tubes represented a minor aggregation state, and conversely. Sampling was done in quadrats of 25 × 25 cm (n with and without macroalgae = 20) selected randomly on the reef surfaces in February 2009. The null hypothesis of no differences in the number of visible tubes between areas with and without macroalgae was evaluated with a tc test (Zar, 1999).

2.4.2. Effects of macroalgae on the growth and body condition of *Ficopomatus F. enigmaticus*

To investigate if changes in macroalgae presence affected the growth and body condition of individuals of *F. enigmaticus*, we transplanted reefs without macroalgae to areas with macroalgae (mainly *P. subtilissima*; warm season: January 2009; cold season: August 2009). In each case, we extracted reef pieces with PVC cylinders (as described in the recruitment experiment; n = 24) in which the ends of the tubes were marked with nontoxic paint. Then, reef pieces were implanted in the center of 12 areas with 100% macroalgae cover, and in 12 areas without macroalgae (area size: 40 × 40 cm) which were randomly selected on different reefs. After 45 days we analyzed individual survival and estimated tube growth as the increment in length from the marked paint (n = 1 tube per experimental unit). We also calculated body condition as previously described. The null hypothesis of no differences in the mean growth and body condition of polychaetes between areas with and without macroalgae was tested with tc tests (Zar, 1999) for both warm and cold seasons.

2.5. Statistical analysis

In repeated measure ANOVAs (Zar, 1999), sphericity in the repeated measures factors was evaluated with Mauchly Sphericity Test; if sphericity could not be met, we performed the Greenhouse–Geisser Epsilon adjustment of the degrees of freedom (see Crowder and Hand, 1999). In ANOVAs, the null hypothesis of equal variances was evaluated with paired Levene tests (Zar, 1999). When variances were heterogeneous, data were transformed; if heterogeneity could not be avoided, we performed an ANOVA on rank transformed data under the null

Table 1
Results of the repeated measures ANOVAs, on range transformed data, comparing the mean abundances of (A) macroalgae and (B) *F. enigmaticus* on *A. brasiliana* valves, among months and valve sides. Hereafter, significant p values (<0.05) are shown in bold.

Abundances	Test for de means			Univariate adjust for repeated measures		
	F	df	p	ϵ	df	p
A) Macroalgae (% cover)						
Months	9.97	4;72	<0.05	0.58	2.34;42.18	<0.05
Valves side	48.64	1;72	<0.05	–	–	–
Months × valves side	9.21	4;72	<0.05	0.58	2.34;42.18	<0.05
B) <i>F. enigmaticus</i> (# of tubes · valve⁻¹)						
Months	2.5	4;72	<0.05	0.71	2.85;51.37	p > 0.05
Valves side	6.16	1;72	<0.05	–	–	–
Months × valves side	2.18	4;72	>0.05	0.71	2.85;51.37	p > 0.05

hypothesis of no differences in mean range values (robust ANOVA to heterogeneity of variances; Quinn and Keough, 2002). Specific mean comparisons were made with Tukey HSD tests on corresponding factors (p < 0.05). When there were no differences in the means, we considered differences between variances (Levene test, Zar, 1999) to discuss the effects of other sources of variability (see Fraterrigo and Rusak, 2008).

3. Results

3.1. Relationship between *Ficopomatus F. enigmaticus* and macroalgae recruitment on bare substrate

In the valves of *A. brasiliana* the mean percent cover of macroalgae (*P. subtilissima* and green macroalgae) varied with months and side of the valves (Table 1). Cover was higher on the external side of the valve in December 2007 and October 2008 (Tukey HSD, month × side, p < 0.05; Fig. 3A). The mean number of tubes of *F. enigmaticus* varied with valve sides (Table 1), being higher on the internal side of the valve (Fig. 3B).

There was no correlation between the macroalgae percent cover and the number of *Balanus improvisus* on the external side of the valves (p = 0.33, p > 0.05), nor between the mean number of crabs and number of tubes of *F. enigmaticus* on the valves (p = 0.31, p > 0.05).

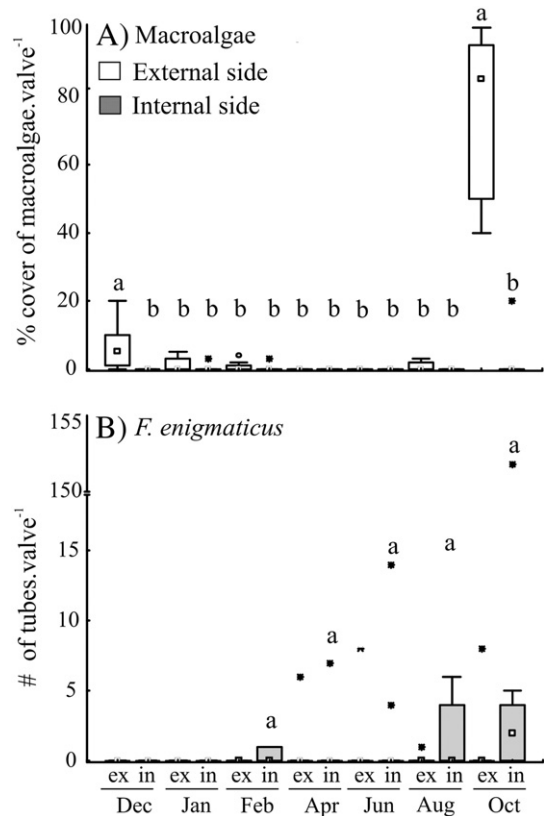


Fig. 3. (A) Percent cover of macroalgae and (B) number of recruits of *F. enigmaticus* on the valves of *A. brasiliana* by months and valve sides (x axis where “in” is internal and “ex” is the external side. Hereafter: symbols within boxes are the median, box limits are 25th and 75th percentiles, bars are 5th and 95th percentiles, circles are values outside 95% of the distribution and asterisks are extreme values; letters show differences among means (Tukey HSD for corresponding factors, p < 0.05).

3.2. Macroalgae effects on recruitment of *Ficopomatus F. enigmaticus*

3.2.1. Description of the recruitment of *Ficopomatus F. enigmaticus* on hard substrates, with and without macroalgae

The abundances of macroalgae (mainly *Cladophora* sp.) and *F. enigmaticus* were different among nuclei categories (one-way ANOVA: macroalgae, 0.17 power transformed data, $F_{3;169} = 12.6$, $p < 0.05$; *F. enigmaticus*: range transformed data, $F_{3;169} = 7.67$, $p < 0.05$). Macroalgae percent cover was higher on the valves of *T. plebeius* and *M. isabelleana* (Tukey HSD, $p < 0.05$; Fig. 4) but the number of tubes of *F. enigmaticus* was higher on the valves of *A. brasiliana* (Tukey HSD, $p < 0.05$; Fig. 4). The abundances of macroalgae and *F. enigmaticus* were different also according the positions in each nucleus category (two-way ANOVA, Table 2). In general macroalgae grew on the side exposed to the sun, while *F. enigmaticus* grew on the internal side of the nuclei. Particularly, the valves of *T. plebeius* showed higher abundance of macroalgae in the convex position (Table 2A; Fig. 5A) while higher abundance of *F. enigmaticus* in the concave-internal position (Table 2B; Tukey HSD for position \times side; Fig. 5A). The valves of *M. isabelleana* showed higher abundance of macroalgae in the convex-external position (Table 2C Tukey HSD, position \times side; Fig. 5B) and an abundance of *F. enigmaticus* near zero, with no differences, and more variability, in the convex-internal position (Table 2D; Levene, $p < 0.05$). The valves of *A. brasiliana* showed higher abundance of macroalgae in the convex-external position (Table 2E; Tukey HSD, position \times side; Fig. 5C) and higher abundance of *F. enigmaticus* in the convex-internal position (Table 2F; Tukey HSD, position \times side; Fig. 5C). Some macroalgae were found on bivalves valves in the semi-buried position (Fig. 5A and B).

3.2.2. Description of the recruitment of *Ficopomatus F. enigmaticus* on reef surfaces with and without macroalgae

The recruitment of *F. enigmaticus* on reef surfaces was higher in areas with macroalgae (mean = 3.17, SE = 0.71) than in reefs without macroalgae (mean = 0.73, SE = 0.47; $t_c = 2.83$, $df = 31$, $p < 0.05$).

3.3. Growth and body condition of *Ficopomatus F. enigmaticus* with and without macroalgae

3.3.1. Description of the growth and body condition of *Ficopomatus F. enigmaticus* with and without macroalgae

The total length of the tubes was higher in areas with macroalgae than in areas without macroalgae in the warm season ($t_c = 1.92$, $df =$

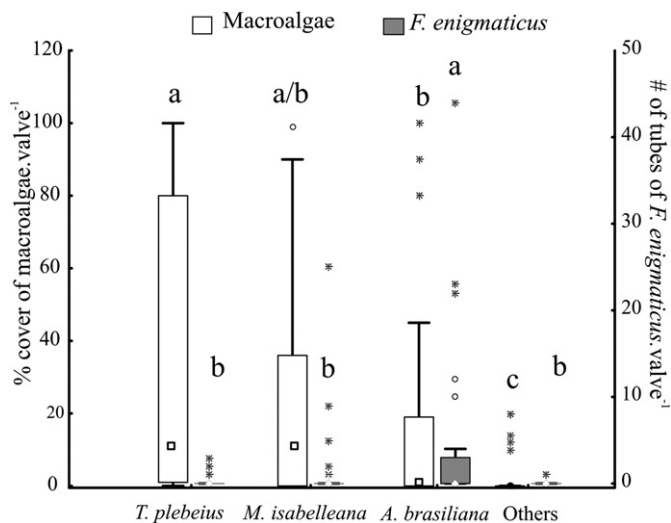


Fig. 4. Macroalgae percent cover (right axis) and number of *F. enigmaticus* tubes (left axis) of the nuclei collected (x axis; n: *T. plebeius*: 40; *M. isabelleana*: 71; *A. brasiliana*: 29; others: 33). Letters show differences among nuclei for macroalgae and *F. enigmaticus* (series).

Table 2

Results of the two-way ANOVAs, on range transformed data, comparing the mean abundance of macroalgae (A, C, and E) and *F. enigmaticus* (B, D, and F) among positions of the different types of valves (*T. plebeius*, *M. isabelleana*, *A. brasiliana*).

Abundances	Test for the means		
	F	df	p
<i>(A) Macroalgae on T. plebeius</i>			
Resting position	20.45	1;27	<0.05
Valve side	4.04	1;27	>0.05
Resting position * valve side	4.04	1;27	>0.05
<i>(B) F. enigmaticus on T. plebeius</i>			
Resting position	33.91	1;27	<0.05
Valve side	42.51	1;27	<0.05
Resting position * valve side	33.91	1;27	<0.05
<i>(C) Macroalgae on M. isabelleana</i>			
Resting position	11.84	1;63	<0.05
Valve side	5.9	1;63	<0.05
Resting position * valve side	5.9	1;63	<0.05
<i>(D) F. enigmaticus on M. isabelleana</i>			
Resting position	1.07	1;63	>0.05
Valve side	0.03	1;63	>0.05
Resting position * valve side	0.03	1;63	>0.05
<i>(E) Macroalgae on A. brasiliana</i>			
Resting position	41.69	1;25	<0.05
Valve side	14.47	1;25	<0.05
Resting position * valve side	14.47	1;25	<0.05
<i>(F) F. enigmaticus on A. brasiliana</i>			
Resting position	14.14	1;25	<0.05
Valve side	12.58	1;25	<0.05
Resting position * valve side	3.65	1;25	<0.05

51, $p < 0.05$, Fig. 6A). In the cold season, however, there were no differences between areas in total length means ($t_c = 0.4$, $df = 64$, $p > 0.05$; Fig. 6B) or variances (Levene, $P > 0.05$). We observed the same pattern with the body condition of *F. enigmaticus*; it was higher in areas with macroalgae than in areas without macroalgae in the warm season ($t_c = 7.14$, $df = 11$, $p < 0.05$; Fig. 6C), but was no different in the cold season ($t_c = -0.12$, $df = 32$, $p > 0.05$; Fig. 6D), and there were no differences in the variances (Levene, $p > 0.05$).

Mean abundance of visible tubes was higher in areas without macroalgae (mean abundance = 3.59, SE = 1.39) than in areas with macroalgae (mean abundance = 0.76, SE = 0.51; $t_c = 8.59$, $df = 24$, $p < 0.05$).

3.3.2. Effects of macroalgae on the growth and body condition of *Ficopomatus F. enigmaticus*

Polychaete transplants showed higher growth in areas with macroalgae than areas without macroalgae in the warm season ($t_c = 3.38$, $df = 22$, $p < 0.05$; Fig. 6A), but not in the cold season ($t_c = 0.85$, $df = 22$, $p > 0.05$; Fig. 6B); the variances did not differ in the cold season (Levene, $p > 0.05$).

The mean body condition of the polychaete transplants showed no differences between areas with and without macroalgae in any season (warm: $t_c = 0.40$, $df = 22$, $p > 0.05$, Fig. 6C; cold: $t_c = -1.49$, $df = 15$, $p > 0.05$; Fig. 6D). The variances in body condition were higher in areas with macroalgae (both seasons; Levene, $p < 0.05$).

4. Discussion

Our results show no evidence of interaction between *Ficopomatus F. enigmaticus* and macroalgae in the colonization of new hard substrates. However, there was higher recruitment of *F. enigmaticus* in areas with macroalgae on reef surfaces. Additionally, polychaete tube lengths and body condition were higher in areas with macroalgae during the warm season.

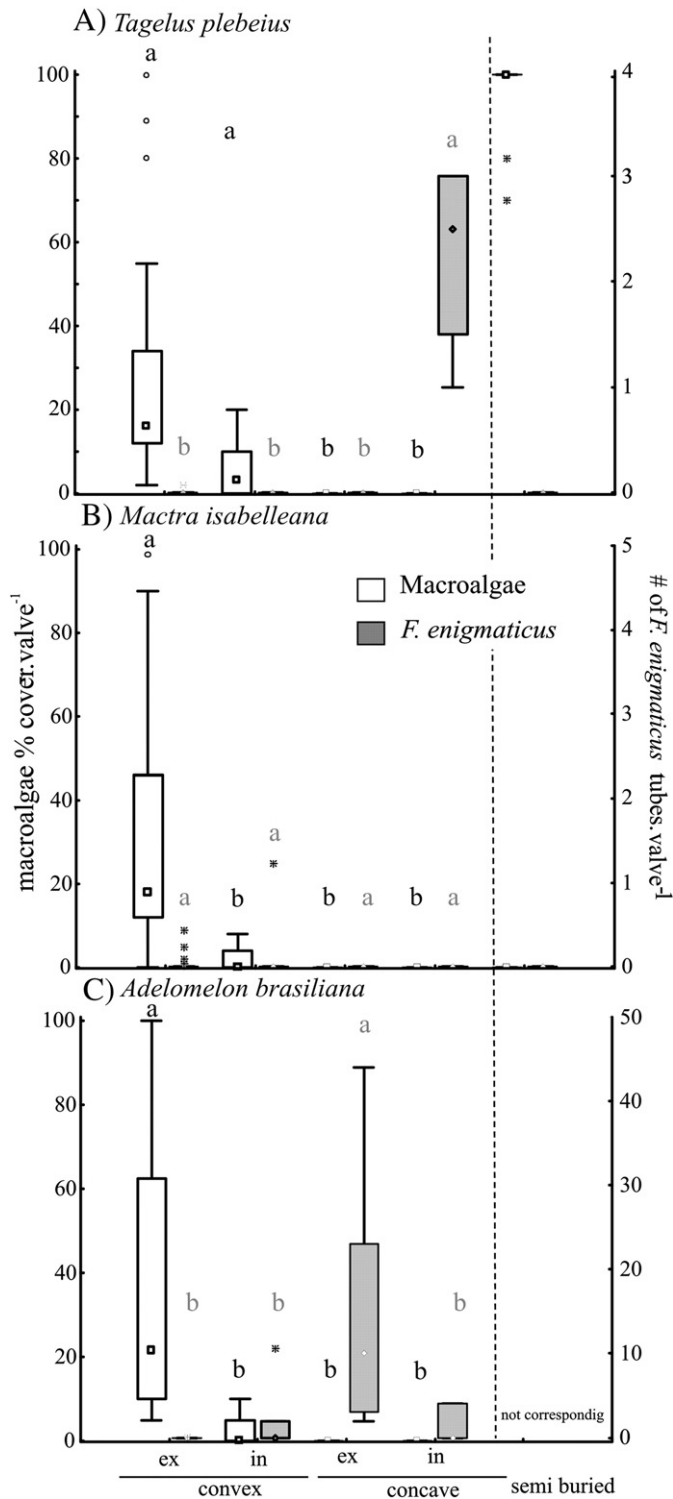


Fig. 5. Percent cover of macroalgae (right axis) and number of *F. enigmaticus* tubes (left axis) on each side of the valves (“in”: internal side, “ex”: external side) among positions (x axis) for valves of (A) *T. plebeius*, (B) *M. isabelleana* and (C) *A. brasiliana*. Black letters show differences among positions for percent cover of macroalgae. Gray letters show differences among positions for numbers of *F. enigmaticus* tubes. In (A) and (B) data for the semi-buried position are shown graphically.

Macroalgae and serpulid polychaetes are able to compete for substrate (Kupriyanova et al., 2001; Paine and Suchanek, 1983) but we observed that macroalgae mainly grew on the external side of the valves while *F. enigmaticus* grew on the internal side of valves of *A. brasiliana*. Different environmental conditions on each side of the

valve, together with different requirements by *F. enigmaticus* and macroalgae, might explain this pattern. For example, inside the valves, a higher availability of refuge from consumers (e.g., the omnivorous crab *Cyrtograpsus angulatus*) might favor *F. enigmaticus* (see Schwindt and Iribarne, 2000), and a low light intensity might limit macroalgae (e.g., light intensity as limiting factor; Thomsen and McGlathery, 2006). Although we did not find a relationship between crab abundance and the number of *F. enigmaticus* recruits inside the valves, consumption should not be discarded as a hypothesis since no exclusion experiments were done. However, even without knowing the mechanisms, our results suggest no interaction between macroalgae and *F. enigmaticus* in the colonization of new substrates.

Macroalgae facilitate the recruitment of *F. enigmaticus* on the reef surfaces. This fact may be a consequence of some physical and chemical modifications induced by macroalgae that could positively affect polychaetes. For example, physical changes could be a decrease in light intensity (see Straughan, 1972), a decrease in water velocity (see Eckman, 1983), or an increase in food availability (see Kupriyanova et al., 2001). Chemical changes could be modifications of the substrate composition (e.g., a higher calcium concentration of the thallus; see Kupriyanova et al., 2001) or the release of macroalgae metabolites into the environment (see Walters et al., 1996). Although mechanisms remain unknown, the effects of macroalgae on the recruitment of *F. enigmaticus* were positive, particularly when macroalgae grew on the reefs.

On reef surfaces, favoring the recruitment of *F. enigmaticus* and promote a greater tube length and better body condition of the polychaetes in warm months. In general, knowledge of the effects of macroalgae growing on polychaete reefs is scarce. Macroalgae could occlude the upper end of the tube of *F. enigmaticus* individuals, limiting food capture (e.g., filter feeders, Denley and Underwood, 1979; Dittman and Robles, 1991) and other processes such as the release of gametes (Wahl, 2008). Therefore, given that *F. enigmaticus* have morphological plasticity (Nishi and Nishihira, 1997), the elongation of the tubes may be a response to minimize these macroalgae effects. However, macroalgae positively affect the body condition of *F. enigmaticus* during the warm season. This pattern could be the result of an increase in food availability (i.e., macroalgae as food traps, see Wahl, 1989) or a reduction in the number of polychaetes feeding in areas with macroalgae. In turn, the low abundance of tubes might also contribute to the tube elongation since tube length is inversely proportional to density (Straughan, 1972). The results suggest that, during warm months, the effects of macroalgae on the reef surfaces would be more important than the colonization of new substrates.

The interaction between macroalgae and *F. enigmaticus* studied here occurred primarily through ecosystem engineering since reef structures create hard substrate, which increases the biomass of macroalgae on its surface (Bazterrica et al., 2012). Therefore, the process can be considered an ecosystem engineering feedback (see Jones et al., 2010), in which the increase of macroalgae positively affects the ecosystem engineer. These effects were less evident in the cold season since the body condition did not change, and the tube elongation with macroalgae was minimal, probably because the abundance of macroalgae is low or null during the cold season (Bazterrica et al., 2012). The macroalgae effect on *F. enigmaticus* would be important in the months of highest algal biomass, months that coincide with the recruitment and higher growth of *F. enigmaticus* (i.e., the warm season).

In contrast to the widely known role of environmental variables on *F. enigmaticus* reef development (e.g., Bianchi and Morri, 2001; Fornós et al., 1997; Schwindt et al., 2004a), further work needs to be done to establish the mechanisms involved in the biological interactions controlling *F. enigmaticus* spread. For example, there is little evidence for control mechanisms such as consumption (e.g., low consumption by fish due to their low abundance, Schwindt and Iribarne, 1998; lack of evidence of consumption by crabs during recruitment into valves of *A. brasiliana*, Bazterrica et al., 2013; Schwindt and Iribarne, 2000).

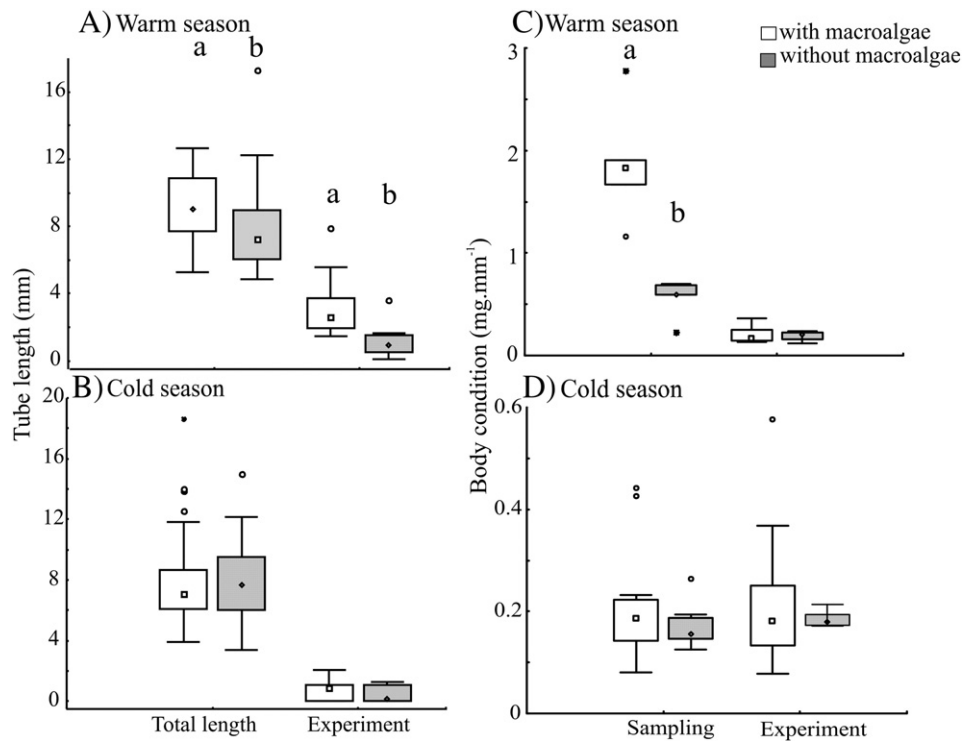


Fig. 6. The lengths of the tubes (y axis), in areas with and without macroalgae on reef surface, during sampling and experiments (x axis), are showed for the (A) warm and (B) cold seasons. The body condition of *F.enigmaticus*, in areas with and without macroalgae on reef surfaces, during sampling and experiments is showed for the (C) warm and (D) cold seasons. Letters show differences in length between areas with and without macroalgae (series) for each variable considered.

Our study provides evidence of a positive feedback for *F. enigmaticus* mediated by macroalgae, affecting established reefs during the higher growth season (i.e., summer). This feedback might favor the growth of the reef structures by allowing the elongation of the tubes, increasing body condition, and favoring recruitment. When there is a lack of evidence of the factors limiting the spread of the populations, those factors affecting the established population could be significant controlling pathways (see McQuaid and Lindsay, 2000; Petraitis, 1995). Therefore, our study contributes to the understanding of invasive processes, highlighting the need to identify the impact of interactions among the species that characterize the invaded community.

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References

Alfaro, A.C., Francois, T., Sergent, L., Duxbury, M., 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuar. Coast. Shelf Sci.* 70, 271–286.

Anderson, M.J., 1999. Distinguishing direct from indirect effects of grazers in intertidal estuarine assemblages. *J. Exp. Mar. Biol. Ecol.* 234, 199–218.

Bazterrica, M.C., Botto, F., Iribarne, O., 2012. Effects of an invasive reef-building polychaete on the biomass and composition of estuarine macroalgal assemblages. *Biol. Invasions* 14, 765–777.

Bazterrica, M.C., Alvarez, M.F., Bruschetti, M., Hidalgo, F., Fanjul, E., Iribarne, O., Botto, F., 2013. Factors controlling macroalgae assemblages in a Southwest Atlantic coastal lagoon modified by an invading reef forming polychaete. *J. Exp. Mar. Biol. Ecol.* 443, 169–177.

Bianchi, C.N., Morri, C., 1996. *Ficopomatus* “reefs” in the Po River Delta (Northern Adriatic): their constructional dynamics, biology, and influences on the brackish-water biota. *PSZN I: Mar. Ecol.* 17, 51–66.

Bianchi, C.N., Morri, C., 2001. The battle is not to the strong: serpulid reefs in the lagoon of Orbetello (Tuscany, Italy). *Estuar. Coast. Shelf Sci.* 53, 215–220.

Borthagaray, A.I., Clemente, J.M., Boccardi, L., Brugnoli, E., Muniz, P., 2006. Potential impact of *Ficopomatus enigmaticus* (Fauvel) (Polychaeta: Serpulidae) invasion in Laguna de Rocha, Uruguay. *Pan-Am. J. Aquat. Sci.* 1, 57–65.

Bouma, T.J., Ortells, V., Ysebaert, T., 2009. Comparing biodiversity effects among ecosystem engineers of contrasting strength: macrofauna diversity in *Zostera noltii* and *Spartina anglica* vegetations. *Helgol. Mar. Res.* 63, 3–18.

Brankevich, G., Bastica, R., Lemmi, C., 1988. A comparative study of biofouling settlements in different sections of Necochea power plant (Quequén port, Argentina). *Biofouling* 1, 113–115.

Bruno, J.F., O'Connor, M.I., 2005. Cascading effects of predator diversity and omnivory in a marine food web. *Ecol. Lett.* 8, 1048–1056.

Charles, F., Jordana, E., Amouroux, J.M., Grémare, A., Desmalades, M., Zudaire, L., 2003. Reproduction, recruitment and larval metamorphosis in the serpulid polychaete *Ditrupea arietina* (O.F. Müller). *Estuar. Coast. Shelf Sci.* 57, 435–443.

Cohen, A.N., Carlton, J.T., 1995. Nonindigenous aquatic species in a United States estuary: a case study of the biological invasions of the San Francisco Bay and Delta. Report for the US Fish and Wildlife Service and Connecticut Sea Grant Program (251 pp.).

Crooks, J.A., 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* 97, 153–166.

Crooks, J.A., 2009. The role of exotic marine ecosystem engineers. In: Rilov, G., Crooks, J.A. (Eds.), *Biological Invasions in Marine Ecosystems. Ecological, Management and Geographic Perspectives*. Springer, Verlag Berlin Heidelberg (642 pp.).

Crowder, M.J., Hand, D.J., 1999. *Analysis of Repeated Measures*. Chapman and Hall, New York (257 pp.).

Daleo, P., Escapa, M., Alberti, J., Iribarne, O., 2006. Negative effects of an autogenic ecosystem engineer: interactions between coralline turf and an ephemeral green alga. *Mar. Ecol. Prog. Ser.* 315, 67–73.

Davies, B.R., Stuart, V., de Villiers, M., 1989. The filtration activity of a serpulid polychaete population (*Ficopomatus enigmaticus* (Fauvel)) and its effects on water quality in a coastal marina. *Estuar. Coast. Shelf Sci.* 29, 613–620.

Denley, E.J., Underwood, A.J., 1979. Experiments on factors influencing settlement, survival, and growth of two species of barnacles in New South Wales. *J. Exp. Mar. Biol. Ecol.* 36, 269–293.

Dittman, D., Robles, C., 1991. Effect of algal epiphytes on the mussel *Mytilus californianus*. *Ecology* 72, 286–296.

- Eckman, J.E., 1983. Hydrodynamic processes affecting benthic recruitment. *Limnol. Oceanogr.* 28, 241–257.
- Fornós, J.J., Forteza, V., Martínez-Taberner, A., 1997. Modern polychaete reefs in Western Mediterranean lagoons: *Ficopomatus enigmaticus* (Fauvel) in the Albufera de Menorca, Balearic Islands. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 128, 175–186.
- Fraterrigo, J.M., Rusak, J.A., 2008. Disturbance-driven changes in the variability of ecological patterns and processes. *Ecol. Lett.* 11, 756–770.
- Isla, F.I., 1997. Seasonal behavior of Mar Chiquita tidal inlet in relation to adjacent beaches, Argentina. *J. Coast. Res.* 13, 1221–1232.
- Jones, C.G., Gutiérrez, J.L., 2007. On the purpose, meaning, and usage of the ecosystem engineering concept. In: Cuddington, K., Byers, J.E., Wilson, W.G., Hastings, A. (Eds.), *Ecosystem Engineers: Plants to Protists*. Academic Press, New York, NY, USA, pp. 3–24.
- Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. *Oikos* 69, 373–386.
- Jones, C.G., Gutiérrez, J.L., Byers, J.E., Crooks, J.A., Lambrinos, J.G., Talley, T.S., 2010. A framework for understanding physical ecosystem engineering by organisms. *Oikos* 119, 1862–1869.
- Kupriyanova, E., Nishi, H.A., Ten Hove, T., Rzhavsky, A.V., 2001. Life-history patterns in serpulimorph polychaetes: ecological and evolutionary perspectives. *Oceanogr. Mar. Biol. Annu. Rev.* 39, 1–101.
- Lubchenco, J., 1982. Effects of grazers and algal competitors on fucoid colonization in tide pools. *J. Phycol.* 18, 544–550.
- McCook, L., Jompa, J., Diaz-Pulido, G., 2001. Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs* 19, 400–417.
- McQuaid, C.D., Lindsay, T.L., 2000. Effect of wave exposure on growth and mortality rates of the mussel *Perna perna*: bottom-up regulation of intertidal populations. *Mar. Ecol. Prog. Ser.* 206, 147–154.
- Nishi, E., Nishihira, M., 1997. Spacing pattern of two serpulid polychaetes, *Pomatoleios kraussi* and *Hydroides elegans* revealed by the nearest-neighbor distance method. *Nat. Hist. Res.* 4, 101–111.
- Obenat, S.M., Pezzani, S.E., 1994. Life cycle and population structure of the polychaete *Ficopomatus enigmaticus* (Serpulide) in Mar Chiquita coastal lagoon, Argentina. *Estuaries* 17, 263–270.
- Paine, R.T., Suchanek, T.H., 1983. Convergence of ecological processes between independently evolved competitive dominants: a tunicate-mussel comparison. *Evolution* 37, 821–831.
- Petraitis, P.S., 1995. The role of growth in maintaining spatial dominance by mussels (*Mytilus Edulis*). *Ecology* 76, 1337–1346.
- Quinn, G.P., Keough, M.J., 2002. *Experimental Design and Data Analysis for Biologist*. Press Syndicate of the University of Cambridge, United Kingdom (527 pp.).
- Rabaut, M., Guilini, K., Van Hoey, G., Vincx, M., Degraer, S., 2007. A bio-engineered soft-bottom environment: the impact of *Lanice conchilega* on the benthic species-specific densities and community structure. *Estuar. Coast. Shelf Sci.* 75, 525–536.
- Read, G.B., Gordon, D.P., 1991. Adventive occurrence of the fouling serpulid *Ficopomatus enigmaticus* (Polychaeta) in New Zealand. *N. Z. J. Mar. Freshw. Res.* 25, 269–274.
- Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, M., Molofsky, J., With, K.A., Cabin, R.J., Cohen, J.E., Norman, C., Mccauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., Weller, S.G., 2001. The population biology of invasive species. *Annu. Rev. Ecol. Syst.* 32, 305–332.
- Schwindt, E., Iribarne, O.O., 1998. Reef of *Ficopomatus enigmaticus* (Polychaeta: Serpulidae) in the Mar Chiquita coastal lagoon, Argentina. *Boll. Soc. Hist. Nat. Balears* 41, 35–40.
- Schwindt, E., Iribarne, O., 2000. Settlement sites, survival and effects on benthos of an introduced reef-building polychaete in a SW Atlantic coastal lagoon. *Bull. Mar. Sci.* 67, 73–82.
- Schwindt, E., Bortolus, A., Iribarne, O.O., 2001. Invasion of a reef-builder polychaete: direct and indirect impacts on native benthic community structure. *Biol. Invasions* 3, 137–149.
- Schwindt, E., De Francesco, C.G., Iribarne, O.O., 2004a. Individual and reef growth of the invasive reef-building polychaete *Ficopomatus enigmaticus* in a south-western Atlantic coastal lagoon. *J. Mar. Biol. Assoc. UK* 84, 987–993.
- Schwindt, E., Iribarne, O.O., Isla, F.I., 2004b. Physical effects of an invading reef-building polychaete on an Argentinean estuarine environment. *Estuar. Coast. Shelf Sci.* 59, 109–120.
- Stewart, H.L., Carpenter, R.C., 2003. The effects of morphology and water flow on photosynthesis of marine macroalgae. *Ecology* 84, 2999–3012.
- Straughan, D., 1972. Ecological studies of *Mercierella enigmatica* (Anellida: Polychaeta) in the Brisbane river. *J. Anim. Ecol.* 41, 93–136.
- Thomsen, M.S., McGlathery, K., 2006. Effects of accumulations of sediments and drift algae on recruitment of sessile organisms associated with oyster reefs. *J. Exp. Mar. Biol. Ecol.* 328, 22–34.
- Thorp, C.H., 1994. Population variation in *Ficopomatus enigmaticus* (Fauvel) (Polychaeta, Serpulidae) in a brackish water millpond at Emsworth, West Sussex, U.K. *Mém. Mus. Nat. Hist. Nat.* 162, 585–591.
- Troost, K., 2010. Causes and effects of a highly successful marine invasion: case-study of the introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries. *J. Sea Res.* 64, 145–165.
- Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Westbrooks, R., 1996. Biological invasions as global environmental change. *Am. Sci.* 84, 468–478.
- Wahl, M., 1989. Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Mar. Ecol. Prog. Ser.* 58, 175–189.
- Wahl, M., 2008. Ecological lever and interface ecology: epibiosis modulates the interactions between host and environment. *Biofouling* 24, 427–438.
- Wallentinus, I., Nyberg, C.D., 2007. Introduced marine organisms as habitat modifiers. *Mar. Pollut. Bull.* 55, 223–332.
- Walters, L.J., Hadfield, M.G., Smith, C.M., 1996. Waterborne chemical compounds in tropical macroalgae: positive and negative cues for larval settlement. *Mar. Biol.* 126, 383–393.
- Zar, J.H., 1999. *Biostatistical Analysis*. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA (663 pp.).