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ORIGINAL PAPER

Effects of nutrient enrichment and grazing by an invasive filter feeder on phytoplankton biomass in a South West Atlantic coastal lagoon

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Abstract Invasive species can exert strong effects on structure and function of marine ecosystem. In estuaries, nutrient enhancement due to anthropogenic activities can increase microalgal biomass but invading filter-feeders can eventually regulate their abundance. This is what we hypothesized that is happening in a SW Atlantic coastal lagoon (Mar Chiquita, 37°40′S, 57°23′W, Argentina) invaded by the polychaete *Ficopomatus enigmaticus*. Here, we experimentally evaluated this hypothesis in a mesocosms experiment. Four treatments were performed: (T1) with nutrients (NO_3, PO_4 and NH_4), (T2) with reefs and nutrients, (T3) with reefs, and (T4) without reefs or nutrients. Water samples were obtained to determine in vivo chlorophyll *a* (Chl*a*) and nutrients concentration. Additionally, to evaluate the trophic position and particle selectivity by the polychaete, analysis of stable isotopes of particulate organic matter (POM) of the water and individuals were performed. Stable isotopes analysis showed that the main resource used by *F. enigmaticus* was the fraction of POM between 62 and 250 μm, showing particle selectivity and suggesting that the reefs have the potential to promote shifts in size, composition and biomass of local food source. Mesocosms experiments showed that nutrient supply increased the Chl*a* concentration, but when reefs were added, the Chl*a* decreased. Thus, nutrients increased the phytoplankton biomass but grazing by the reefs counteracted these increments. This indicates that an invader such as *F. enigmaticus* might modulate an anthropogenic impact via suspension feeding, and therefore its role in mitigate the consequences of eutrophication may be highly important.

Keywords Filter-feeders · Nutrients · Top-down and bottom-up effect · Invasive reef-building polychaete · Grazing · Eutrophication

Introduction

Biological invasions are one of the main threats to the functioning of the world ecosystems (Vitousek 1990; Mack and D'Antonio 1998; Carlton 2000). Marine invaders can have profound impacts on ecosystem processes or services (Grosholz et al. 2009; Gutiérrez et al. 2014) and one important challenge for ecologists is

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to distinguish invaders with lower effects from those with large effects in order to prioritize management efforts (Parker et al. 1999). However, less attention has been placed on potential positive effects of the invaders on the ecosystem functions (see Guy-Haim et al. 2018) and how these effects might be manifested in the benefices that marine ecosystems provide to humans.

The relative importance of the nutrients (bottom-up control) and the consumers (top-down control) on the structure and regulation of communities of coastal ecosystems has been discussed extensively in the literature (Micheli 1999; Heck et al. 2000; Valiela et al. 2004). In aquatic ecosystems, non-native grazers can directly regulate the abundance of pelagic primary producers and have indirect cascading effects on food webs (Alpine and Cloern 1992; Strayer 2010; Sousa et al. 2014). Top-down control of phytoplankton biomass by invasive suspension feeders is expected to increase ecosystem resiliency to changes in nutrient inputs with a simultaneous potential to control eutrophication (Gottlieb and Schweighofer 1996). In contrast, nutrient enrichment can generate an increase of the primary producer biomass and consequently increase the system productivity (Valiela et al. 2000; Teichberg et al. 2010). Nutrient loading is one of the main consequences of intensive human land use at the land-sea interface (Valiela 2006; Teichberg et al. 2010). The human population along coastline is growing and the associated wastewater has become one of the primary sources of nitrogen, and consequently it has increased primary production in estuaries and coastal waters (Cloern 2001; Valiela 2006). Usually, bottom-up and top-down processes operate simultaneously (Alpine and Cloern 1992; Valiela et al. 2004) and their relative influence on ecosystem productivity may differ depending upon the environment and its inherent productivity (Burkepile and Hay 2006). Therefore, understanding what processes control the productivity of natural ecosystems, and what the ecological role of the invasive species in this ecosystem function is an important challenge in marine ecology.

In the Mar Chiquita coastal lagoon (37°40′S, 57°23′W, Buenos Aires, Argentina) the primary production is regulated by nutrient availability via continental water input (De Marco et al. 2005; Marcovecchio et al. 2005). The Mar Chiquita's basin have approximately one million hectares and includes a large portion of the flooding pampas and southern Argentine pampas (Soriano et al. 1992). It combines the major regional land uses, including traditional agriculture, mixed agricultural-livestock systems and pure livestock systems in the lower fields (Booman et al. 2012). The lagoon acts as a sink for many of the streams in the basin, and consequently concentrates large amounts of nutrients in the water (Marcovecchio et al. 2005). Although the required causes for the eutrophication of this lagoon exist (i.e., a relatively stable hydrological regime with low hydrodynamic exchange; Lanfredi et al. 1987, and input of inorganic nutrients; Marcovecchio et al. 2005), signs of hyper-eutrophication process (e.g., nuisance blooms, anoxia, macroalgae or epiphyte growth, increase of supply of organic matter) have never been observed. The Mar Chiquita coastal lagoon is one of the places in the world more threatened by the invasion of the reef-forming polychaete *F. enigmaticus* (Schwindt and Iribarne 2000; Schwindt et al. 2004). *Ficopomatus enigmaticus* has successfully invaded this lagoon since 1970s and has generated strong changes in the sedimentation patterns, and in the native benthic community structure (Schwindt et al. 2001). This invasive polychaete decreases the phytoplankton biomass of the lagoon and their grazing effect has been postulated as a control of primary production (see Bruschetti et al. 2008; Pan and Marcoval 2014). Therefore, joint regulation of phytoplankton biomass by nutrient input and grazing may be expected. We hypothesized that an invader might modulate an anthropogenic impact (i.e., eutrophication) via suspension feeding.

In this study, the trophic position of this invasive polychaete and its food sources were studied by stable isotope analysis from individuals and water samples. Then, we aimed to understand the relative importance of bottom-up and top-down controls on primary producers with the presence of an invasive filter-feeder polychaete. We experimentally investigated the grazing effect of the invasive reef-forming polychaete *F. enigmaticus* and the increase of nutrients on phytoplankton biomass of the lagoon.

Materials and methods

Study area

The experiment was performed at the Mar Chiquita Coastal Lagoon (an UNESCO Man and the Biosphere Reserve; Fig. 1) during April of 2010. The lagoon is a brackish water area of 46 km^2 permanently connected to the sea (Isla 1997). The entrance channel is approximately 6 km long, 200 m wide and its depth is between 1.5 and 2 m (Fasano et al. 1982). It is characterized by small tidal amplitudes (up to 1 m; Isla 1997) which are mainly generated by the winds and basin discharge. The area where the experiment was performed (Paraje San Gabriel, Fig. 1) is about six km from the lagoon inlet. The reefs of *F. enigmaticus* occupy large areas at the innermost zone of the lagoon, reaching up to 370 reefs ha^{-1} and up to 7 m in diameter (mean density = 89 reefs ha^{-1}, average diameter = 3.7 m, Schwindt et al. 2004).

Food source and trophic position: stable isotope analysis

Stable isotopes have also been used to define food webs within estuaries (Peterson and Fry 1987). Because elements such as carbon (C) and nitrogen

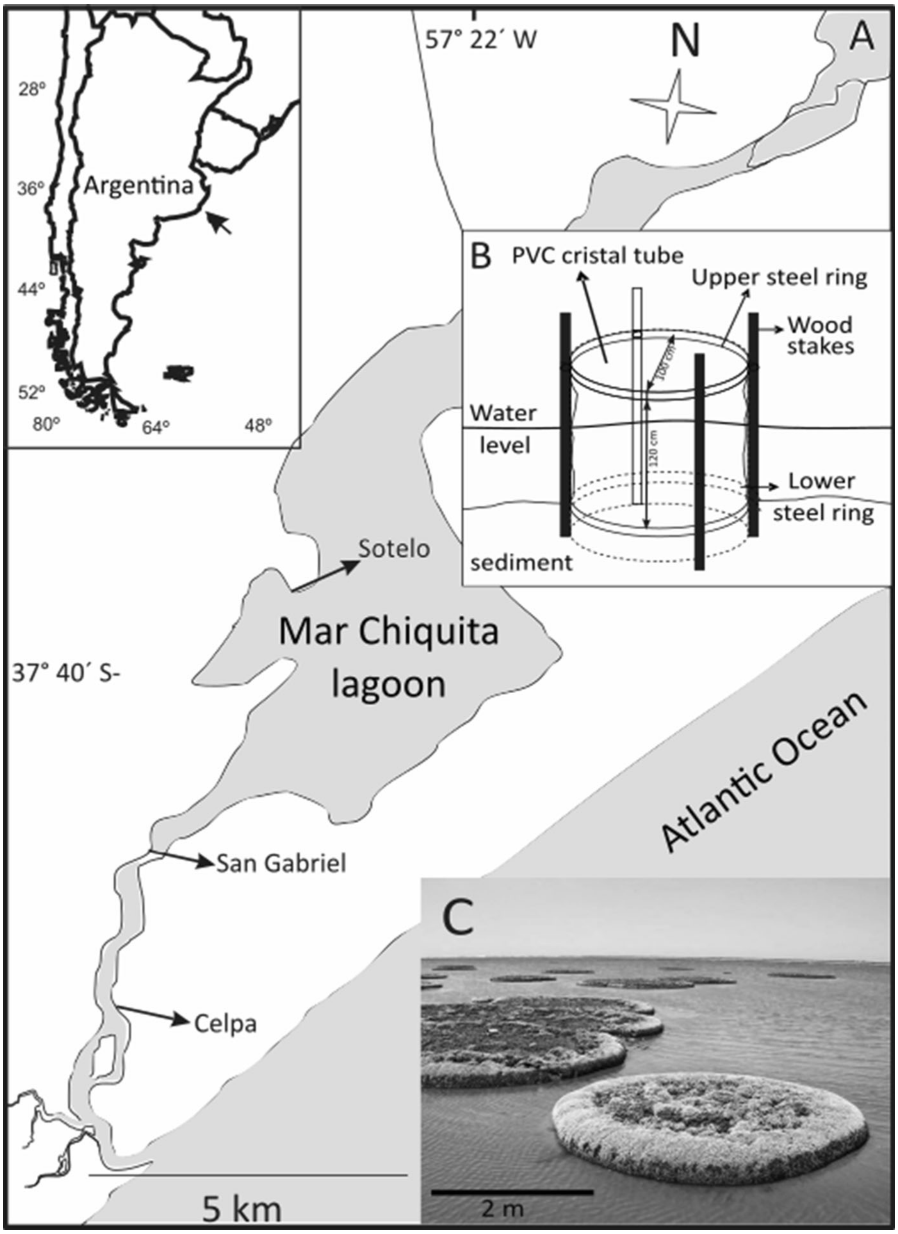

Fig. 1 *a* Map of Mar Chiquita lagoon (Buenos Aires, Argentina) showing the sites of sampling and experimenting, *b* schematic drawing of a mesocosms, *c* picture of the reefs of *F. enigmaticus* in Mar Chiquita lagoon

(N) fractionate in predictable ways in aquatic organisms, it is possible to construct food webs based on these data. The ratio of $^{15}N/^{14}N$ is used to determine trophic position. ^{15}N in consumer tissues is enriched in about a 3–4‰ respect to those of its prey (Fry and Sherr 1984). On the other hand, ^{13}C values show a slower fractioning with an increase of 0–1‰ for each trophic level, but they are closely related to organic matter origin (Fry and Sherr 1984; Peterson et al. 1985). Because of this stepwise trophic level enrichment, N and C stable isotopes have become a valuable tool in food web analysis.

To determine the trophic position and which fraction of POM is preferentially selected by *F. enigmaticus,* we evaluated the isotopic signal of three fractions of water (> 250 µm, between 250 and 62 µm, < 62 µm) and the polychaetes from three areas of the lagoon: Celpa (Ce), Sotelo (So) and San Gabriel (SG; see Fig. 1a). The fractions of water were chosen to cover the entire estuarine phytoplankton size spectrum. The first fraction (< 62 µm) principally contains picoplankton and nanoplankton. The second fraction (between 62 and 250 µm) is mainly composed of microplankton, and the third fraction (> 250 µm) of mesoplankton. The study areas have decreasing salinities (So < SG < Ce), an increase of water turbidity (So > SG > Ce; Isla 1997) and likely different sources of carbon due to the proximity of the water sources (freshwater or marine; see Fig. 1a). Samples of individuals were randomly taken in different reefs (n = 5) and areas inhabited by *F. enigmaticus*. The individuals were extracted from its tubes, put into tin capsules, dried 24 h at 70 °C, ground to fine powder and weighed. Due to the methodology and the need to obtain a determined weight for each sample, worms (n = 10) from the different reefs were pooled. To obtain three different fractions of POM, samples of water (n = 5, volume = 10 l) were taken in the three study areas, and filtered through two sieves of decreasing mesh size (250 and 62 µm). The samples of POM in Mar Chiquita are composed largely of organic matter from producers (i.e., phytoplankton; Botto et al. 2005). Afterwards, the material trapped in the sieves and the discarded volume was filtered in a low-pressure vacuum with Whatman GF/C glass-fiber filters previously ashed at 500 °C (diameter = 47 mm, pore size = 1.2 µm). The filters were stored at − 25 °C prior to analysis. Isotope analysis was performed by a mass spectrometer in the stable isotope facility of the University of California Davis. The stable isotope ratios are expressed as δ values as $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$, where R is the corresponding ratio $^{15}N/^{14}N$ o $^{13}C/^{12}C$.

Differences in the percentages of $\delta^{15}N$ and $\delta^{13}C$ in the polychaete *F. enigmaticus* between areas were evaluated with a one-way ANOVA. The MixSIAR Bayesian isotope mixing model in R (Stock and Semmens 2013) was used to identify which fraction of water of the lagoon (POM) is consumed by the filter-feeder polychaete. Mixing models use isotopes stables data to estimate the proportions of source (prey) contributions to a mixture (consumer). Bayesian mixing models improve upon simpler linear mixing models by explicitly taking into account uncertainty in source values and prior information (Moore and Semmens 2008; Stock and Semmens 2013). The input data of the MixSIAR model were the isotope values ($\delta^{15}N$ and $\delta^{13}C$) from the individuals of *F. enigmaticus* (consumers) and from the three fraction of POM (sources; data as means and SDs) of each area. We used 3.4‰ (SD = 1) as the trophic fractionation for $\delta^{15}N$ and 0.4‰ (SD = 1.3‰) for $\delta^{13}C$. These values of trophic fractionation were obtained using data from the literature of multiple aquatic ecosystem studies, and even though variable, are robust and widely applicable (see Post 2002). The Markov Chain Monte Carlo parameter was set to “short” run length. The error structure was specified in the model as “residual only”. Trace plots and the diagnostic tests Gelman–Rubin, Heidelberger–Welch, and Geweke were used to determine whether the model converged or not.

Mesocosms

To evaluate the grazing effect of the invasive polychaete, the increase of nutrients and interactive effects between both variables on pelagic primary productivity we performed in situ mesocosm experiments (n = 4) with four treatments: (T_1) with nutrients, (T_2) with reefs and nutrients, (T_3) with reefs, and (T_4) without reefs or nutrients (control). The experiment ran for 7 days and the days of sampling were April 23, 26, 28 and 30 of 2010. The mesocosms were built in clear PVC crystal tubes (500 µm thickness, 95% translucence) of 100 cm diameter and 110 cm height (see Fig. 1b). The upper open end was attached to a steel ring with plastic seals, and was kept at about 50 cm above the maximum tidal level. The open lower border was also attached to a steel ring and buried 30 cm into the sediment. The mesocosms were localized at 20 m from the coastline in an area where the reefs naturally occur. Three equally sized reef fragments (40 × 40 cm, 30 cm height) taken from naturally occurring *F. enigmaticus* reefs (see Fig. 1c) were included in each of the eight mesocosms (T_2 and T_3). A treatment consisting of reefs without worms (dead reefs) to control for the hydrodynamic effects of reefs was not considered here because water flow is expected to be negligible inside mesocosms. Given that, the evaluation of the number of live polychaetes in each mesocosms is a destructive method; we have assumed that similar sizes of reefs have similar number of live polychaetes.

To deliver nutrients into the mesocosms (T_1 and T_2), we installed two perforated plastic bottles (volume = 500 ml, diameter = 6 cm, height = 23 cm) in each enclosure fixed upright in the borders of the mesocosms and in the middle of the water column (40 cm from the sediment). The bottles were filled with 1M KNO_3, 1M NH_4Cl and 1M $Na_2HPO_4–2H_2O$ dissolved in a 3% agar solution (following Martinetto et al. 2011), and pierced with a screwdriver (20 holes per bottle). Control mesocosms contained bottles with agar solution only. This method has been previously tested and it ensured a slow and constant release of nutrients. The employed volume (500 ml of agar) guaranteed the effective nutrients diffusion during the 7 days of experimental running (Bazterrica et al. 2013). Also, prior to starting the experiments, we measured nitrate (NO_3), phosphate (PO_4) and natural ammonium concentration (NH_4) in the water column of Mar Chiquita coastal lagoon. Then, based on the ambient concentration of nutrients in the lagoon (Marcovecchio et al. 2005; Bazterrica et al. 2013), we calculated the level of N and P addition that represented a considerable increase (3×) relative to the ambient concentration per mesocosms. The increase was within the range of fertilization values observed in other experiments (see Worm et al. 2000) and represented a realistic increase in nutrient loading found in other estuaries (see Cloern 2001).

Water samples were taken every 2–3 days (April 23, 26, 28 and 30 of 2010) with 60 ml syringes from mesocosms (day 0, day 3, day 5 and day 7) and out of mesocosms (n = 4; Out). Nitrate, phosphate and ammonium were measured in filtered samples through a glass microfiber GF/F filter and frozen until analysis at the end of the experiment. Nutrients were determined using standard colorimetric methods, phenol hypochlorite method for NH_4 (Solorzano 1969), cadmium reduction method for NO_3 and Strickland and Parsons 1972 method for PO_4. The in vivo chlorophyll *a* concentration (μg l^{-1}) was determined in and out of mesocosm with an Aquafluor handheld Fluorometer (Turner Design, Model 8000–01. Detection range: 0.05–300 μg Chl*a* l^{-1} and 0.5–150 NTU).

The initial physical conditions of the water (i.e., pH, conductivity, turbidity, dissolved oxygen, temperature and salinity) and biological parameters (chlorophyll *a*) were assumed equal because the mesocosms were mounted in the same place and time (i.e., we installed all mesocosms simultaneously to ensure similar initial conditions). The mesocosms were not sealed during the experimentation. Therefore, the water of the mesocosms was partially renewed every day because there was water exchange through the bottom of the sediment of the enclosures.

To evaluate if there were differences in baseline concentration of nutrients (NO_3, PO_4 and NH_4) and Chl*a* between treatments, all parameters were measured 2 h before the beginning of the addition of nutrients and/or reefs inside the mesocoms and analyzed with a two way ANOVA (factors: nutrients and reef). There were no baseline differences in Chl*a* and PO_4 concentration between the different treatments (see Table S1 in Supplementary material). In contrast, baselines of NO_3 concentration were affected by nutrient addition and the NH_4 was interactively affected by factors of nutrient addition and presence of reefs (Table S1). Tukey test showed that baseline NO_3 concentration was higher (12%) in fertilized treatments than in unfertilized treatments ($P < 0.05$). However, in the 3rd, 5th and 7th day of sampling the increase was up to 300, 494 and 432% respectively, and therefore the differences in baseline concentration were negligible.

To evaluate the separate and interactive effects of reefs, nutrient addition and time on the phytoplankton biomass (in vivo Chl*a*) and nutrients of the water of the mesocosm, we performed a mixed-effect model with the lme function from the nlme package (Pinheiro et al. 2013) in R 3.0.1 (R Development Core Team 2013). Nutrients, reefs and time were treated as fixed factors, and mesocoms were treated as the random factor. To account for the lack of independence among time-series observations, we modeled dependence of these data using autoregressive correlation structure of order 1, following Pinheiro and Bates (2000). The final model was selected using the likelihood ratio test (always consistent with the AIC criterion) following Zuur et al. (2009). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality.

In addition, to evaluate differences in nutrients and phytoplankton concentration of the water between mesocosms control (T_4) and open water (out), we performed a mixed-effect model with the same function as above-mentioned. No artifact effects were found for NH_4, NO_3 and PO_4, given that mesocosms and open water only differed one single (and different) day each (day 5 for NH_4, day 3 for PO_4 and day 7 for NO_3; see Table S2 and Fig. S1 in Supplementary material). Although there were some contrasting differences in Chl*a* between mesocosms and the open water in different dates (mesocosms had higher Chl*a* in day 0, lower in day 3 and higher again in day 5), the overall difference (including the whole experiment; $X_{control} = 25.7$ (SD = 10.9), $X_{out} = 21.7$ (SD = 13.7) was negligible. Moreover, the largest differences were found between dates, and they simulated the main experiment (see Table S2, Fig S1).

Results

Food source and trophic position: stable isotopes analysis

Isotopic signal of the individuals of *F. enigmaticus* from the three different areas showed differences in their values of $\delta^{13}C$ ($F_{2,\ 12} = 87.7$, $P < 0.001$; Fig. 2), possibly indicating different use of carbon sources in the lagoon. The N-isotopic values showed differences between the individuals of Celpa and the others sites ($F_{2,\ 12} = 15.2$, $P < 0.001$; Fig. 2). Values of San Gabriel and Sotelo were marginally different ($t = -2.3$, $P = 0.08$).

In our work, POM isotopes values ranged between − 24.1 and − 20.4‰ for $\delta^{13}C$ and 8.5 and 5.2‰ for $\delta^{15}N$. Estuarine POM carbon isotope values fluctuated between freshwater POM (− 26) and estuarine phytoplankton (approx. − 21‰; see Botto et al. 2005). C-isotopic values from the individuals of *F. enigmaticus* overlapped with the signal of the sources (POM, Fig. 2), showing values between − 23.6 and − 19‰.

Median values from the posterior distributions of Bayesian isotope mixing models suggested that the fraction of POM between 62 and 250 μm (hereafter: fraction *b*) was the main resource used for *F. enigmaticus* (see Table 1) followed by fraction > 250 μm (hereafter: fraction *c*) and fraction < 62 μm (hereafter: fraction *a*) in all sites.

The median diet for individuals from Sotelo creek was 70.5% represented for the fraction *b*, 14.5% for the fraction *c*, and 5.2% for the fraction *a*. In San Gabriel, the median diet of the polychaetes was 83.7, 9.7 and 3.3% and, in Celpa was 72.3, 18.1 and 6.4% represented for the fraction *b*, *c* and *a* respectively.

Mesocosms

Nutrients

Our results showed that the experimental nutrient enrichments successfully increased nutrient concentration above control treatments and ambient concentration. There was an interactive effect between factors of nutrient and time (N × T) in the concentration of NO_3 and NH_4 (Table 2; Fig. 3). NO_3 concentration was higher in fertilized treatments in the 3rd ($T = 2.54$, $P < 0.05$; Fig. 3c), 5th ($T = 4.76$, $P < 0.001$) and 7th ($T = 2.56$, $P < 0.05$) day of experimentation. NH_4 concentration was higher in treatments with nutrients in the 3rd ($T = 6.19$, $P < 0.001$) and 5th ($T = 6.59$, $P < 0.001$; Fig. 3a) day of experimentation. The average concentration of NH_4, and NO_3 was up to 51 and 3.2 time higher in the experimental nutrient enrichments than in no-enrichment treatments respectively.

The concentration of PO_4 in the mesocosms was affected by nutrient addition and time, but not by the interaction between them. The fertilized mesocosms had nine time higher concentration of PO_4 than in the mesocosms with ambient concentration ($t = 6.15$, $P < 0.001$; Fig. 3b). The concentration of PO_4 was not consistent in the experimental time, being in the 3rd day higher than in the 5th ($t = 4.02$, $P < 0.005$) and 7th day ($t = 1.86$, $P < 0.05$), but the 5th day had lower concentration than in the last day of experimentation ($t = -2.17$, $P < 0.05$).

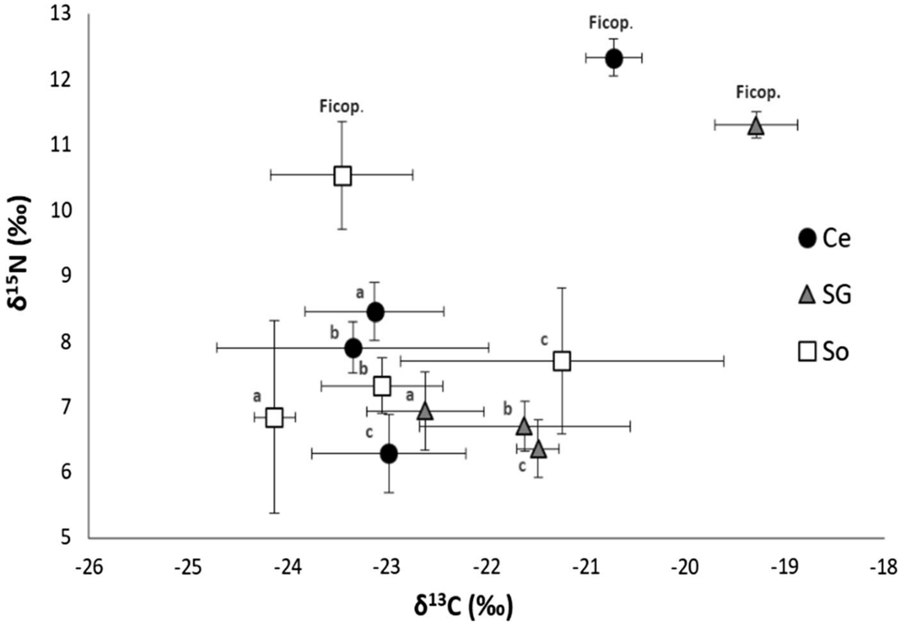

Fig. 2 $\delta^{15}N$ versus $\delta^{13}C$ (mean ± SD) for individuals of *F. enigmaticus* (Ficop.) and three different fractions of POM (*a* < 62 µm, *b* between 250 and 62 µm, *c* > 250 µm) from Mar Chiquita lagoon in Celpa (black circle), San Gabriel (grey rhombus) and Sotelo creek (white square). The fraction b was the main resource used for *F. enigmaticus* for each site

Table 1 Mean, quantiles (50, 2.5 and 97.5%) and SD of proportions of each fraction of particulate organic matter in the diet of *F. enigmaticus* (*a* < 62 µm, *b* between 250 and 62 µm, *c* > 250 µm) from Celpa, San Gabriel and Sotelo

	Celpa			San Gabriel			Sotelo		
	a	b	c	a	b	c	a	b	c
Mean	0.1	0.64	0.26	0.08	0.7	0.21	0.13	0.6	0.26
SD	0.10	0.28	0.24	0.11	0.30	0.26	0.18	0.32	0.27
2.5%	0.002	0.05	0.004	0.001	0.02	0.001	0.001	0.02	0.001
50%	0.064	0.723[a]	0.181	0.033	0.837[a]	0.097	0.052	0.705[a]	0.145
97.5%	0.38	0.98	0.80	0.42	0.99	0.73	0.68	0.99	0.99

The quantiles 2.5 and 97.5% are Bayesian credible intervals

[a] The median source contribution value for each area

Chlorophyll a concentration

Our results showed that both nutrients addition and presence of reefs of *F. enigmaticus* affected interactively the Chl*a* concentration in the mesocosms (N × R; Fig. 4, Table 2). Post hoc test showed that the treatment with reefs and nutrients (T_2) had lower concentration of Chl*a* (t = – 4.4, $P < 0.001$) compared with the treatment with only nutrients (T_1; X = 19.4), indicating that the reefs decreased the Chl*a* concentration in fertilized mesocosms (Table 2, Fig. 4). Although there were not differences in the Chl*a* concentration between treatments (with and without reefs) in mesocosms with ambient nutrients (Table 2, Fig. 4), the concentration of Chl*a* was lower with reefs than without reefs on 3rd and 5th day. However, not difference was found at 7th day. In addition, the treatments with only nutrients had higher concentration of Chl*a* compared with control treatments (t = 1.86, $P < 0.05$), showing that the experimental nutrient enrichments successfully increased Chl*a* concentration above ambient concentration. Chl*a* was affected by time (Table 2), being different during all days and increasing through the experimental time ($P < 0.001$; Fig. 4). This increase was the same as out of mesocosms (see Fig. S1d), indicating that it was not an artifact effect but a feature of the water in the experimental time.

Table 2 Results of linear mixed-effects models evaluating the effect of nutrients, reefs, and time on the Chl*a*, NO_3, PO_4 and NH_4 concentration of the water of the mesocosms in the Mar Chiquita lagoon

Source of variation	*df*	*F*	*P*
Chlorophyll *a*			
Nutrients (N)	1. 16	0.19	0.66
Reef (R)	1. 16	16.76	< 0.001*
Time (T)	1. 16	218.1	< 0.001*
N × R	1. 16	4.7	< 0.05*
N × T	2. 32	0.56	0.57
R × T	2. 32	0.15	0.85
N × R×T	2. 32	2.66	0.09
NO_3			
Nutrients (N)	1. 16	11.4	< 0.005*
Reef (R)	1. 16	2.28	0.15
Time (T)	2. 32	30.8	< 0.001*
N × R	1. 16	2.14	0.16
N × T	2. 32	20.23	< 0.001*
R × T	2. 32	1.42	0.25
N × R×T	2. 32	0.17	0.84
PO_4			
Nutrients (N)	1. 16	37.8	< 0.001*
Reef (R)	1. 16	1.14	0.3
Time (T)	2. 32	8.11	< 0.005*
N × R	1. 16	1.01	0.32
N × T	2. 32	1.8	0.18
R × T	2. 32	1.73	0.19
N × R×T	2. 32	1.77	0.18
NH_4			
Nutrients (N)	1. 16	60.2	< 0.001*
Reef (R)	1. 48	2.89	0.09
Time (T)	2. 32	10.32	< 0.001*
N × R	1. 48	3.17	0.08
N × T	2. 32	9.86	< 0.001*
R × T	2. 48	0.044	0.95
N × R × T	2. 48	0.008	0.99

Nutrients, reef and time were treated as fixed factors, and mesocosms was treated as a random factor

*Significant differences

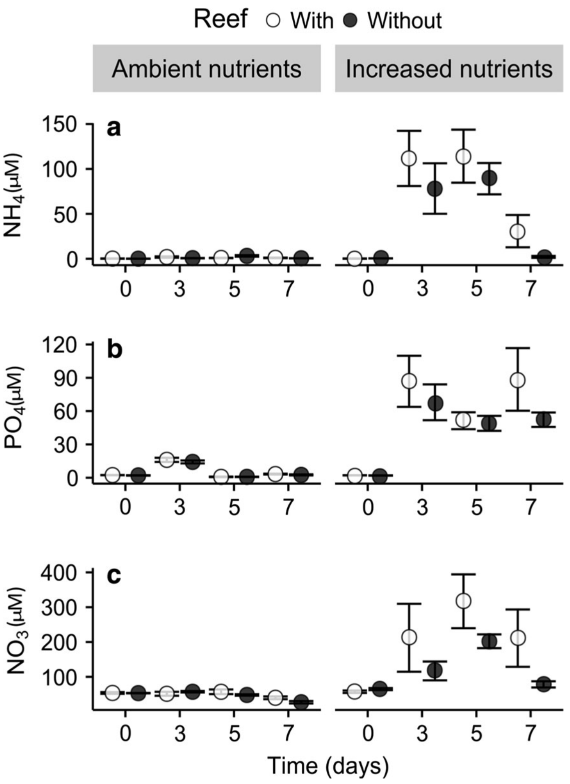

Fig. 3 Effect of nutrients, time and reef addition on **a** ammonium (μM), **b** phosphate (μM), **c** nitrate (μM) concentration of the water of the mesocosms in Mar Chiquita coastal lagoon

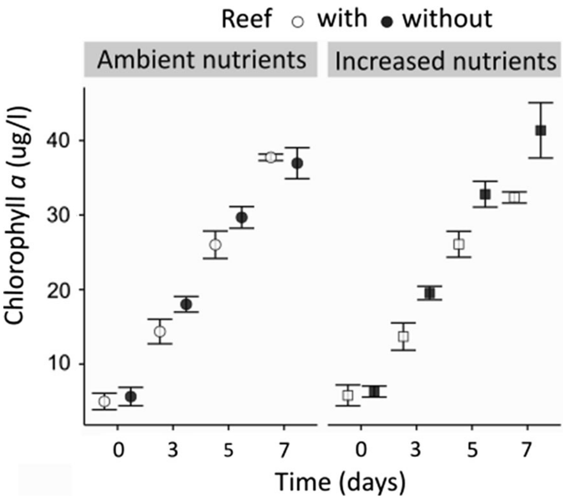

Fig. 4 Effect of nutrients, time and reef addition on chlorophyll a (μg/) concentration of the water of the mesocosms in Mar Chiquita coastal lagoon

Discussion

Stable isotope analysis showed particle selectivity by *F. enigmaticus* suggesting that the reefs have the potential to promote shifts in size, composition and biomass of plankton communities of invaded systems. The results showed that both top-down and bottom-up processes are important forcing factors that control the biomass of primary producers in this site. In fact, when nutrients increased the biomass of pelagic primary producers, reefs counteracted these increases.

Studies about food sources (diet) of invasive species are necessary to understand how invaders affect diversity and availability of food sources for other species (i.e. trophic overlap and eventually competition). Our results show that the trophic position of *F. enigmaticus* is typical of a filter-feeder species. Our work show that the main resource used by this invasive polychaete was the fraction of particules between 62 and 250 µm, which mainly includes the microplankton (between 20 and 200 µm). Microplankton is composed of several heterotrophic and autotrophic taxonomic groups. Diatoms (centric and pennate) dominate the microplanktonic assemblage in this water body (88% of the total cell counts; Pan and Marcoval 2014). Clearance rate of *F. enigmaticus* for pennate diatoms was higher than for any other planktonic prey (2.3 l h^{-1} g DW^{-1}; Pan and Marcoval 2014) and, therefore, this phytoplankton fraction could be their main food source and more affected. Considering the clearance rate calculated in situ (Pan and Marcoval 2014) and the total water volume of the lagoon (about 2.76×10^{10} l based on 46 Km^2 area and 0.6 m mean depth; Fasano et al. 1982), the polychaetes are able to filter all the lagoon water in almost 6 days (140 h). Although these calculations have many assumptions (e.g., equal filtration rates for polychaetes independently of their size, clearance rates that do not vary with particle concentration, no water renewal at the lagoon) and therefore are just approximation, they gives a good idea to the potential magnitude of the grazing effect of this invasive polychaete.

The large range of C-isotopic values of the polychaetes (− 24 to − 19‰) from three areas of the lagoon suggests the influence of different sources of carbon consumed. In estuaries, POM is a mixture of terrestrial and marine components, consisting of phytoplanktonic cells, organic debris, and eroded material from soils and sediments (Riley et al. 2004). In Mar Chiquita lagoon, phytoplankton, microalgae and marsh plants debris (i.e., the cordgrass *Spartina densiflora*) all contribute to the organic matter of the estuarine food web (Botto et al. 2005). However, the filter-feeders are more dependent of the material in the column water, and our results show this pattern. Phytoplankton assemblages might vary in time and space, and changes in species composition may explain some of the variation in $\delta^{13}C$ (see Cifuentes et al. 1988). However, the main influence of freshwater or marine sources in the three areas studied could be determinant in the isotopic signal of the individuals.

Trophic position of the invasive polychaete was different to the other main native filter-feeder (i.e., the razor clam *Tagelus plebeius*; see Botto et al. 2005) that inhabit in the study area (Addino 2014). *Tagelus plebeius* had values of $\delta^{13}C$ (approx. − 18) intermediate between signals of deposit feeders species (whose main resource is debris of *S. densiflora* and benthic microalgae) and filter-feeders species (Botto et al. 2005). In our work *F. enigmaticus* had values of $\delta^{13}C$ near to pelagic sources (i.e., phytoplankton; see Botto et al. 2005). Although both species are the main local benthic filter feeders, the native razor clam takes food (water) from the surface layer near to the bottom, and the invasive polychaete from the water column. However, the resuspension caused by local action of wind or tide of sedimentary material, may considerably mixing the seston available to both suspension-feeders (Dubois et al. 2009). Thus, the non-native filter feeder *F. enigmaticus* would affect the diversity, composition and biomass of local food source (seston) to other native grazer.

High densities of low-trophic position taxa, such as grazers, are predicted to have strong effects on energy pathways of invaded ecosystems (Capps et al. 2015; Cucherousset et al. 2012). *Ficopomatus enigmaticus* is a non-native grazer with high density and biomass (Schwindt et al. 2004), and has affected ecosystem functions of the invaded system in past decades by control primary production, increasing water clarity (Davies et al. 1989; Bruschetti et al. 2008) and also increasing faunal diversity (Schwindt and Iribarne 2000; Heiman and Micheli 2010). Other ecosystem-level effects of the introduction of this physical ecosystem engineer such as habitat forming (e.g., Heiman et al. 2008; Bruschetti et al. 2009) and alteration of water flows (e.g., Schwindt et al. 2004), are also important. Considering that eutrophication can be defined as an increase in the rate of supply of organic matter to an ecosystem (sensu Nixon 2012), our work show that this invasive polychaeta can counteract this increment.

Input of inorganic nutrients are one of the main causes of eutrophication process (Valiela 2006), but it is not the phenomenon itself. The definition of eutrophication in coastal marine systems can link to the supply of organic matter they receive, rather than to the input of a particular inorganic nutrient (Nixon 2012). The response of pelagic primary producers to nutrient load increase in coastal marine ecosystems differs widely because biological control mechanisms of the eutrophication process are not always the same (Pérez-Ruzafa et al. 2002). Grazing by filter feeders can be a very efficient control mechanism by removing excess biomass generated by excess nutrients (Herman and Scholten 1990). However, removing phytoplankton has a direct effect on nutrient load as they uptake inorganic nutrients from water column (Pérez-Ruzafa et al. 2002). Our findings showed that the reefs of *F. enigmaticus* did not affect the nutrients load of the lagoon, but decreased the phytoplankton biomass (i.e., main component of particulate organic matter of the column water; Botto et al. 2005) in fertilized mesocosms. Although in ambient nutrient condition the concentration of Chl*a* was not consistently lower with reefs, the Chl*a* concentration was lower with reefs than without in 2 days of sampling. In addition, all studies show that the reefs of *F. enigmaticus* decrease the chlorophyll biomass at ambient nutrient conditions (Davies et al. 1989; Bruschetti et al. 2008, 2011; Pan and Marcoval 2014) but this work show that at high levels of nutrients this effect is probably higher. The worldwide coastal nutrients input is growing due to intensifying human land use (Vitousek 1994; Valiela 2006) and our results suggest that at least in this case an introduced species (probably also due to human intervention) is potentially controlling the secondary effects of higher nutrient influx.

In summary, our results show particles selectivity by *F. enigmaticus* suggesting that this invasive polychaete has the potential to promote shifts in size, composition and diversity of the plankton community of invaded systems (see too Pan and Marcoval 2014). Furthermore, *F. enigmaticus* substantially reduced algal biomass when nutrients were added, and therefore its role in mitigate eutrophication may be highly important. All evidences (Davies et al. 1989; Bruschetti et al. 2008; Pan and Marcoval 2014) showed that *F. enigmaticus* is capable to exert a strong top-down control on the pelagic ecosystem of shallow aquatic systems. Our findings provided a strong experimental evidence that when the phytoplankton biomass is increased by nutrients, an invasive polychaete can counteract this effect.

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