

Top-down and bottom-up regulation in a high nutrient–high herbivory coastal ecosystem

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ABSTRACT: In this study, carried out in San Antonio Bay (Northern Argentinean Patagonia), we aimed to understand the relative importance of bottom-up and top-down controls on macroalgal blooms in a macrotidal system with high nutrient supply and high consumer abundance. Our results show that nutrients, pH, and O₂ concentrations were higher during low tide. A field experiment showed that the biomass accumulation rate of *Ulva lactuca* ranged from 6 to 12% d⁻¹ and was reduced by herbivory by 60%. The biomass accumulation rate did not differ in thalli with different initial internal nutrient pools. There was a negative relationship between the percentage of algae consumed and the N content in algal tissues, suggesting compensatory feeding by herbivores. Herbivory reduced the biomass accumulation rate of *U. lactuca* when PO₄³⁻ or no nutrients were added, but not when NO₃⁻ was added. In the absence of herbivory, the addition of nutrients did not increase *U. lactuca* biomass accumulation rate. These results suggest that nutrients remain high enough for adequate time intervals to be assimilated by macroalgae and support blooms. Large water exchange during tidal changes, however, can diminish the potential negative effects of macroalgal accumulation (oxygen depletion, high ammonium concentrations) on herbivores such that herbivores can have a large impact on macroalgae.

KEY WORDS: Top-down vs. bottom-up controls · Coastal eutrophication · Tidal influence · *Ulva lactuca* · Nutrients · Herbivory

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INTRODUCTION

One of the main assumptions in coastal eutrophication studies is that eutrophication causes a significant shift in benthic communities along with great losses of both species and functional diversity (Smith & Schindler 2009). Ultimately, these changes are detrimental for the whole ecosystem and its goods and services (McGlathery et al. 2007).

The most common cause of eutrophication is the addition of nutrients that are mainly derived from land (Nixon 1995, Cloern 2001, Valiela 2006). Increases in nutrient loads typically lead to higher primary production, with consequent changes in the composition and

abundance of flora and fauna (e.g. Duarte 1995, Borum & Sand-Jensen 1996). When eutrophication is incipient, the growth of macroalgae may be positive due to short-term sequestration of nutrients (Howarth et al. 1996, Boyer et al. 2002), providing abundant food particles of high nutritional quality for consumers (Hemmi & Jormalainen 2004). As eutrophication progresses, the accumulation of macroalgae may have negative effects on the system (Smith & Schindler 2009). Macroalgae consume oxygen at night, driving hypoxic events. During the day, the oxygen produced by photosynthesis can restore the oxygen concentration in the water, but a succession of a few cloudy days may prevent this, resulting in near-bottom water becoming

anoxic (D'Avanzo & Kremer 1994). Moreover, bacterial decomposition of macroalgal biomass consumes oxygen (Viaroli et al. 1996), which can cause periods of extended and persistent anoxia. These events of hypoxia and anoxia affect the survival of many organisms such as fishes, shellfishes, and other consumers, including macroalgal grazers (D'Avanzo & Kremer 1994, Fox et al. 2009). Thus, environments with high nutrient loads are characterized by the dominance of fast-growing seaweeds that leads to hypoxic and anoxic events in sediments and near-bottom waters and, consequently, lower abundance and diversity of organisms.

Under this scenario of high nutrient supply and reduction of consumer (including grazer) abundance, most of these systems become bottom-up controlled and top-down control may be negligible (Carpenter et al. 1985, Menge 1992, Raffaelli et al. 1998). In fact, with the exception of rocky shores (Lubchenco 1978, Lotze & Worm 2000), most evidence from coastal areas in temperate latitudes suggests that nutrients are responsible for macroalgal blooms (Sfriso et al. 1992, Valiela et al. 1992, Raffaelli et al. 1998, Burkepile & Hay 2006, Teichberg et al. 2008). In these areas, N is the most common nutrient limiting growth of producers (Ryther & Dunstan 1971, Howarth 1988). Phosphorus can also be limiting for macroalgal growth at some times of the year (Peckol et al. 1994), in N-rich environments (Teichberg et al. 2010) or in carbonate-rich tropical environments (Lapointe et al. 1992).

The effect of nutrient supply on coastal ecosystems may be modified or mediated by hydrodynamics. Most eutrophic sites are subject to low water exchange that concentrate nutrients, as well as photosynthetic products (Cloern 2001). Under high tidal exchange, nutrients may be swept quickly out to sea (Howarth et al. 1996), and hypoxia may be prevented. If the residence time within an estuary is short, primary producers might have insufficient time to grow in response to the nutrient supply within that particular estuary (Monbet 1992, Pace et al. 1992). In addition, free-living or ephemeral attached macroalgae with high transportation potential are much more abundant in meso and microtidal estuaries where the residence time is longer and the hydrodynamic forcing is less (Flindt et al. 2004). Thus, hydrodynamics may control algal growth by limiting the time of exposure to nutrients and, concomitantly, the internal nutrient content in thalli that determines growth rate (Pedersen & Borum 1996). In addition, herbivores may consume different amounts of algae depending on the nutrient content in the algal tissues (Cruz-Rivera & Hay 2000, Giannotti & McGlathery 2001). Consequently, both bottom-up and top-down processes could be affected by hydrodynamics.

The Argentine Patagonian coast is currently subjected to increasing N loading (Teichberg et al. 2010). The human population is growing and the associated wastewater has become one of the primary sources of N, given the lack of agricultural activities and thus low fertilization input. The absence of wastewater treatment plants has led to episodes of eutrophication near human settlements. San Antonio Bay (see Fig. 1) reflects recent changes along the Patagonian coastline due to increased human population size and high nutrient loading. This is a system with high nutrient concentrations ($\approx 121 \mu\text{M}$ for DIN and $\approx 5 \mu\text{M}$ for PO_4^{3-} , Teichberg et al. 2010) that are similar to those found in the central basin of the Venice lagoon (Solidoro et al. 2010), which is a recognized highly eutrophic site.

San Antonio Bay is subject to a semidiurnal symmetric macrotidal regime (up to 9 m) that leads to a large flushing of water twice a day. This considerable water exchange partly relieves the land-derived N loads delivered to San Antonio Bay. Besides the large exchange of water, there are areas with high macroalgal biomass and diversity near the town of San Antonio Oeste (see Fig. 1; Martinetto et al. 2010). The average growth velocity of *Ulva lactuca* at this site is 20 to 25 % d^{-1} , which is 2 to 5-fold higher than those reported in some eutrophic sites around the world (Mondego estuary, Venice lagoon, and Urias estuary; Teichberg et al. 2010). The abundance of consumers is also high in San Antonio Bay (Iribarne et al. 2003, Martinetto et al. 2010), but there are notable differences, with larger populations of consumers in areas closer to human activity where release of nutrients is higher (Martinetto et al. 2010). The abundance of benthic and epibenthic invertebrates and shorebirds is higher in this area. Thus, macroalgal blooms in this system are coupled to a larger abundance of organisms, rather than to a limited survival of consumers. We conjecture that the strength of top-down and bottom-up forces under conditions of high nutrient loads, high growth rates of macroalgae, and high herbivore abundances that are unlimited by oxygen concentrations, should be different from what has been previously shown.

In this paper, we report on the relative importance of bottom-up and top-down controls on macroalgae in San Antonio Bay, a macrotidal system where nutrient supply and consumer abundance are both unusually high. For this purpose, we carried out 2 investigations to evaluate (1) whether land derived nutrients, pH, and O_2 concentrations increased during low tide and were diluted during high tide, and whether tidal channels of San Antonio Bay nearer to human activity differ in nutrient concentrations, isotopic signatures and internal nutrient pools of algal thalli from those farther from human settlement, and (2) whether different internal nutrient pools in thalli or external NO_3^- or PO_4^{3-} supply

and the presence or absence of grazing resulted in differences in biomass accumulation rates of *Ulva lactuca*.

MATERIALS AND METHODS

Study site. Water quality variables were measured in 2 tidal channels of San Antonio Bay: one running nearby and along the town of San Antonio Oeste (hereafter SAO channel; Fig. 1) and another running parallel to the first, but separated from it by a long bar that breaks the second channel's contact with populated areas (hereafter control channel). The bay is 80 km² in area and is characterized by an extensive intertidal (mainly sand/pebble flats) surrounded by salt marshes (mainly *Spartina alterniflora*; Isacch et al. 2006). Given the low average rainfall (~250 mm yr⁻¹; see Isacch et al. 2006), there is almost no freshwater input to the bay via precipitation, making the bay generally hypersaline (Pascual et al. 2001). Septic systems from dwellings along the coast furnish the major source of freshwater to this system via groundwater. This freshwater input is N-rich and affects the SAO channel where high DIN concentrations and canopies of opportunistic fast-growing seaweeds have been reported (Martinetto et al. 2010, Teichberg et al. 2010). Another source of land-derived nutrients is a fish processing factory located on the shore in the innermost part of the SAO channel.

Although we compared nutrient concentrations, algal internal nutrient pools and stable isotopic signatures of C and N between samples obtained from the 2

channels, we did not have site replication, given that truly independent site replicates in nature are virtually impossible to find. However, several similar studies have been published (e.g. Geertz-Hansen et al. 1993, McClelland & Valiela 1998). This is a limitation imposed by the nature of the system that we cannot solve; hence, we assume limitations of our inference to other systems imposed by the lack of site replication. The manipulative experiments were designed to evaluate the response of *Ulva lactuca* growing in a system with high nutrient concentrations and high herbivory, so all the experiments were performed in the SAO channel.

Links among water quality, *Ulva lactuca* N and P internal pools, macroalgal cover, and grazer abundance.

To determine whether there were differences in water quality between low and high tide and between channels, we measured NH₄⁺, NO₃⁻, PO₄³⁻, salinity, pH, and O₂ concentrations in the water at both tidal channels. Access to the control channel was possible only during low tide, while the SAO channel was sampled during both low and high tides. Water samples were taken with 60 ml syringes (6 replicates per tide and channel). To cover yearly fluctuations, measurements were done every 2 to 3 mo between March 2006 and March 2008. Nutrients were determined using standard colorimetric methods—phenolphthalein method for NH₄⁺ (Solorzano 1969), cadmium reduction method for NO₃⁻ (Strickland & Parsons 1972), and Strickland & Parsons' (1968) method for PO₄³⁻. Salinity, pH, and O₂ were measured *in situ* using a multivariable probe (Horiba U-10). Differences in nutrients and chemical variables among high and low tide in the SAO channel and low tide in the control

channel (total of 3 locations) over time (total of 9 dates) were evaluated using 2-way ANOVA for each dependent variable. When an interaction between factors was significant, Tukey tests were applied to determine how factors interacted.

To assess whether the differences in nutrients in the ambient water result in different nutrient contents and isotopic signatures in macroalgal thalli, we determined C, N, and P internal pools and δ¹³C and δ¹⁵N in *Ulva lactuca* thalli collected in the channels at the sites where nutrient samples were taken. To cover seasonal variation, samples were collected in the 2 channels in August and November 2007 for winter and spring, and in January and April 2008 for summer and fall respectively. We collected 3 composite samples (3 to 5 thalli each) per sampled month and

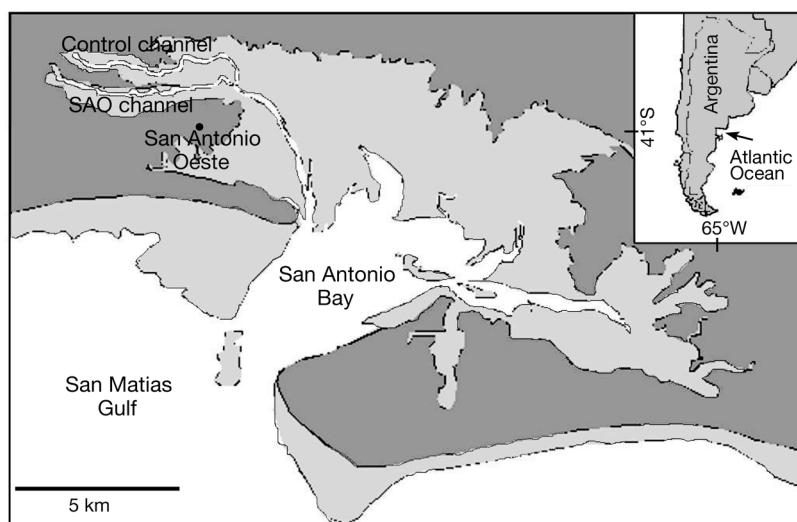


Fig. 1. San Antonio Bay showing flooded area during high tide (light grey) and area under water during low tide (white). The San Antonio Oeste (SAO) channel passes through the town of San Antonio Oeste while the control channel is situated farther away from human activities

channel. The thalli were rinsed with deionized water, dried in an oven at 60°C, ground to a fine powder, weighed, and loaded into tin capsules. Determination of C and N percentages and stable isotope analyses were performed by mass spectrometry in the Stable Isotope Laboratory at The Ecosystems Center, Marine Biological Laboratory (Woods Hole, MA). Phosphorus content was measured in summer and fall samples using colorimetry after extraction of PO_4^{3-} (McGlathery et al. 1994) from thalli. Differences in nutrient contents and ratios as well as in isotopic signatures among seasons and channels were evaluated using 2-way ANOVA for each dependent variable.

To evaluate whether the concentration of nutrients in the ambient water was related to algal cover, we performed regression analyses between percent algal cover and the concentrations of NH_4^+ , NO_3^- , and PO_4^{3-} determined at low tide. We also evaluated the relationship between percent algal cover and the differences in O_2 concentration between low and high tide and the percent increment in $[\text{H}^+]$ at high tide. Proton concentration was calculated as: $[\text{H}^+] = 10^{-\text{pH}}$. Algal cover data was obtained from a previous study (Martinetto et al. 2010) in which percent algal cover was determined visually every 2 to 3 mo over a year (total of 5 dates) from ten 1 m-side quadrats placed randomly in the low intertidal area where chemical variables were measured. Significance of regressions was tested using the *F*-test.

As data on the abundance of herbivorous amphipods and small snails is available from a previous study (Martinetto et al. 2010), we only compared the abundance of chitons and limpets in the present study. Based on data from a food web study carried out in this area (P. Martinetto unpubl. data), we knew that chitons (*Chaetopleura isabellei*) and keyhole limpets (*Fissurella* spp.) were the main macroalgal consumers together with amphipods and small snails. To determine herbivore abundance, twenty 25 cm-side quadrats were randomly surveyed, and all the chitons and keyhole limpets inside the squares were counted. Differences in the abundances of grazers between sites were evaluated using *t*-tests.

Relative effects of internal nutrient pools and herbivory on *Ulva lactuca* growth. To evaluate the relative effects of internal nutrient pools and herbivory on *Ulva lactuca* growth, we experimented on algae with different N and P contents, and with and without herbivory. Thalli were collected from both channels. Three thalli from the same channel were weighed—blotted wet wt = 5.2 ± 1.0 (SD) g cage⁻¹—and placed inside cages (20 × 20 × 15 cm) constructed with transparent Plexiglas (0.5 cm thickness) with 2 sides covered by mesh allowing water flow (Teichberg et al. 2008). Two mesh sizes (1 and 10 mm) were used to

exclude or admit small herbivores. Use of this type of incubation cage in previous studies of algal growth in different coastal areas around the world showed no cage effect (Teichberg et al. 2010).

The experiment consisted of 2 factors: initial internal pool of nutrients (low: control channel; high: SAO channel) and herbivory (with or without herbivores). Eight replicate cages for each treatment combination were set 2 m apart, hanging in a line within the existing macroalgal canopy in the SAO channel. After 12 d of field incubation, algae inside the cages were weighed. We measured the biomass accumulated by *Ulva lactuca* from initial and final weights of algae within each cage. Exponential biomass accumulation rates were determined as: biomass accumulated (g d⁻¹) = (ln final wet wt – ln initial wet wt)/days of incubation.

During the incubation, the algae may incorporate or release nutrients. Since growth is related to the internal nutrient pool in the thalli, we examined changes in the internal nutrient content. The thalli from each cage were also dried and ground for the determination of C and N contents and isotopic signatures as an indicator of the turnover rates of these elements. Phosphorus content was measured as described earlier. Initial C, N, and P contents as well as stable isotopic signatures of C and N were measured in 5 samples consisting of 3 to 5 thalli each, which were collected from both channels. Differences in biomass accumulation rates, nutrient contents, and isotopic signatures among treatments were evaluated using 2-way ANOVA, with initial internal nutrient pool and herbivory as factors.

To evaluate whether consumption by herbivores was related to nutrient content in algal thalli, we performed regression analyses between the percentage of algae consumed in each cage in the treatments with herbivory, and the percentage of N, C, and P and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the thalli. The percentage of algae consumed was calculated as: [(estimated final wt without herbivory – final wt with herbivory) × 100] / estimated final wt without herbivory. The final weight without herbivory was estimated using the mean biomass accumulation rate in the 1 mm mesh cages.

Bottom-up vs. top-down effects on net growth of *Ulva lactuca*. To evaluate the relative impacts of NO_3^- and PO_4^{3-} supply and herbivory on the proliferation of *U. lactuca*, a second experiment was conducted using the same type of incubation cage. In each cage, 3 thalli of *U. lactuca* from the SAO channel were incubated under different treatments combining nutrients and herbivory. The experiment had a cross-classified 2 factor design with 3 treatments of nutrient supply (NO_3^- addition, PO_4^{3-} addition, no nutrient addition), and 2 herbivory treatments (with and without herbivory) (6 treatments, 4 replicate cages for treatments without

herbivory and 8 for those with herbivory). To deliver N and P to macroalgal thalli in the cages, NO_3^- and PO_4^{3-} solutions were prepared by dissolving KNO_3 and KH_2PO_4 in a 3% agar solution, making a 2 M KNO_3 or 1 M KH_2PO_4 solution. Perforated PVC pipes were filled with the agar and placed at the center of each cage to allow for slow release over the course of the experiment. To manipulate the presence and absence of herbivores, we used different mesh sizes (1 or 10 mm) as described above in the first experiment. After 12 d, the incubation chambers were removed and the algae inside the cages were weighed. Differences in the biomass accumulation rates among treatments were evaluated using 2-way ANOVA for unbalanced data (Shaw & Mitchell-Olds 1993) since the number of replicates differed among treatments.

Although the effectiveness of nutrient delivery of this method has been tested (Teichberg et al. 2008), we evaluated the effectiveness of the nutrient addition by sampling water inside and outside (ambient water) the cages on incubation days 2, 6, 9, and 11. Differences in nutrient concentrations among different mesh sizes and nutrient addition treatments were pooled for the incubation period and evaluated using 2-way ANOVA.

RESULTS

Links among water quality, *Ulva lactuca* N and P internal pools, macroalgal cover, and grazer abundance

Concentrations of NH_4^+ , NO_3^- , and PO_4^{3-} were generally higher during low tide in the SAO than in the control channel or during high tide in the SAO channel (Fig. 2a–c). However, there were interactions between factors (location and time) in the concentration of nutrients (interactions: $F_{16,96} = 4.071$, $p < 0.001$; $F_{16,96} = 3.601$, $p < 0.001$; and $F_{16,96} = 2.569$, $p < 0.001$ for PO_4^{3-} , NH_4^+ , and NO_3^- , respectively). Tukey tests revealed that there were no differences in the concentrations of PO_4^{3-} in January 2008, NH_4^+ in June 2007 and March 2008, and NO_3^- in November 2006.

pH and O_2 concentrations were also higher during low tide than during high tide in the SAO channel and higher than those during low tide in the control channel (Fig. 2d,e; factor location: $F_{2,60} = 58.309$, $p < 0.001$; $F_{2,60} = 12.434$, $p < 0.001$ for pH and O_2 respectively) with no variation over time. Salinity was not different between high and low tide in the SAO channel or

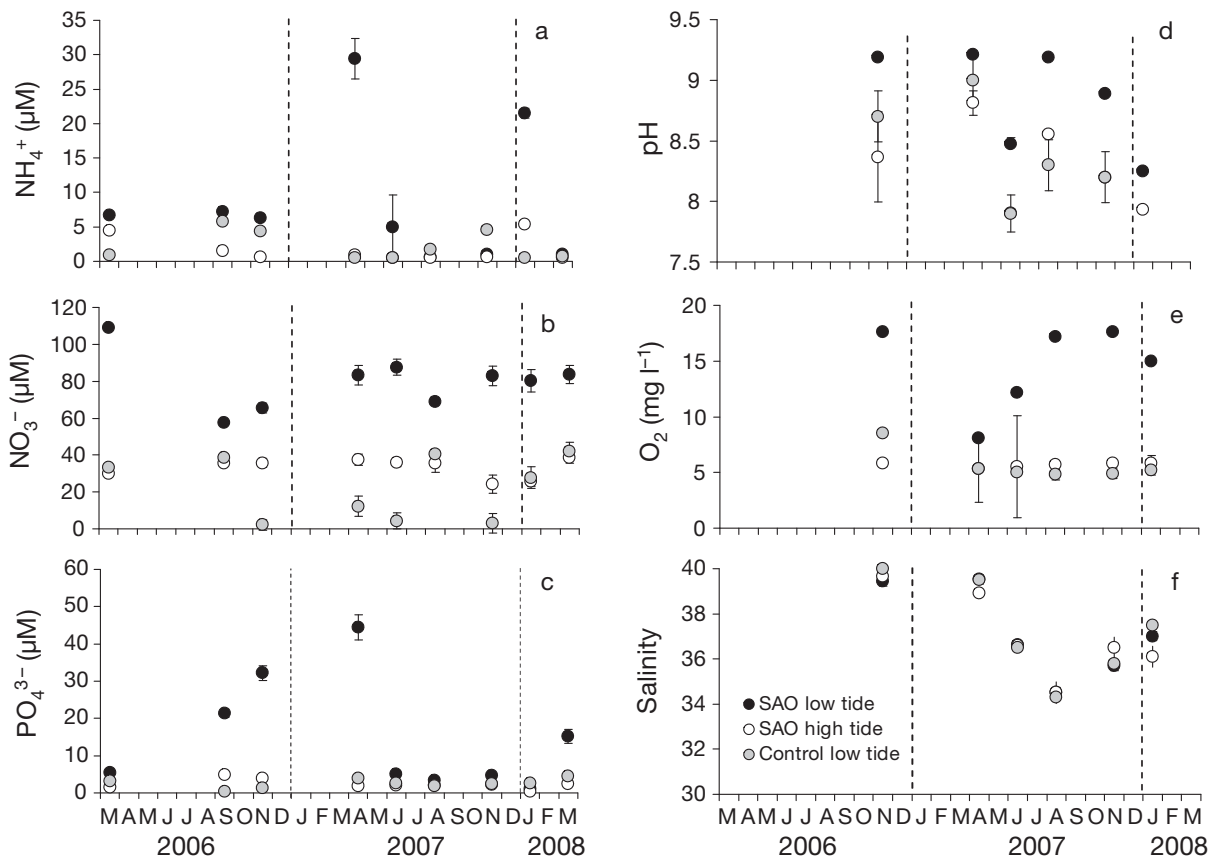


Fig. 2. Mean concentrations (±SE) of (a) NH_4^+ , (b) NO_3^- , and (c) PO_4^{3-} and (d) pH, (e) O_2 , and (f) salinity in the San Antonio Oeste (SAO) channel during high and low tide and in the control channel during low tide

between channels or over time (Fig. 2f; factor location: $F_{2,60} = 34.268$, $p = 0.790$; factor time: $F_{2,60} = 14.385$, $p = 0.928$).

Carbon content was higher in thalli collected from the SAO channel than in those collected from the control channel during spring, summer and fall, while there were no differences between locations in winter (Table 1). The percent N in thalli of *Ulva lactuca* showed interaction between factors (Table 1B), with higher percent N in thalli collected from the SAO than in those from the control channel except for thalli collected in summer which did not show differences between locations (Table 1A). The percent P was higher in thalli from the SAO than in those from the control channel and it was also higher in fall (Table 1). The C:N ratio showed interaction between factors (Table 1B), with higher values in the control than in the SAO channel in winter, spring, and fall and no difference between locations in summer (Table 1A). C:P and N:P ratios were higher in the control than in the SAO

channel, and they were also higher in summer than in fall (Table 1). $\delta^{15}\text{N}$ signatures were higher in thalli from the SAO than in those from the control channel (Table 1). $\delta^{13}\text{C}$ signatures showed interaction between factors (Table 1B), with higher signatures in the SAO than in the control channel over the year, except in winter when values were not different between locations (Table 1A).

Macroalgal cover was negatively related to NH_4^+ concentration (Fig. 3a), but not to NO_3^- or PO_4^{3-} concentration (Fig. 3b,c). It was also positively related to changes in H^+ and O_2 concentrations between tides (Fig. 3d,e).

Similar to the published abundance data on amphipods and small snails (Martinetto et al. 2010), the density of chitons and limpets was higher in the SAO than in the control channel ($t = 2.145$, $p = 0.006$). Density of chitons in the SAO channel was $22.93 \text{ ind. m}^{-2}$ (SE = 5.082) while that in the control channel was 0.53 ind. m^{-2} (SE = 0.363). Keyhole limpets were only present in the SAO channel ($20.27 \text{ ind. m}^{-2}$, SE = 12.223).

Table 1. *Ulva lactuca*. (A) Mean (\pm SE) percentages of C, N, and P content, C:N, C:P, and N:P ratios, and C and N isotopic signatures in thalli from two tidal channels of San Antonio Bay: one near San Antonio Oeste town (SAO) and the other farther away from human activity (control). Percentage of P was measured only in summer and fall. (B) The respective 2-way ANOVA results for each of these variables, with season and location as main factors

(A) Means \pm SE						(B) ANOVA results			
Variable	Location	Winter	Spring	Summer	Fall	Source	df	F	p
%C	Control	34.2 \pm 0.8	33.8 \pm 0.2	34.8 \pm 0.2	30.1 \pm 0.8	Season	3	21.5	< 0.001
	SAO	35.0 \pm 0.6	36.0 \pm 0.4	36.2 \pm 0.2	31.8 \pm 2.3	Location	1	6.8	< 0.001
%N	Control	2.2 \pm 0.2	2.2 \pm 0.2	3.5 \pm 0.0	2.2 \pm 0.5	Interaction	3	0.8	0.512
						Error	37		
	SAO	4.5 \pm 0.2	3.5 \pm 0.2	3.5 \pm 0.1	3.7 \pm 0.8	Season	3	2.3	< 0.001
						Location	1	45.6	< 0.001
%P	Control			0.01 \pm 0.0	0.02 \pm 0.0	Interaction	3	6.96	0.001
						Error	37		
	SAO			0.14 \pm 0.0	0.20 \pm 0.0	Season	1	7.7	0.024
						Location	1	101.4	< 0.001
C:N	Control	18.3 \pm 1.5	18.7 \pm 2.2	11.6 \pm 0.1	14.4 \pm 1.7	Interaction	1	15.8	0.051
						Error	8		
	SAO	9.2 \pm 0.3	12.4 \pm 0.6	12.3 \pm 0.3	7.7 \pm 0.2	Season	3	4.7	0.007
						Location	1	55.6	< 0.001
C:P	Control			764.4 \pm 30.0	661.8 \pm 19.1	Interaction	3	9.1	< 0.001
						Error	37		
	SAO			263.1 \pm 11.2	221.2 \pm 8.3	Season	1	9.5	0.015
						Location	1	404.9	< 0.001
N:P	Control			77.0 \pm 2.3	48.4 \pm 6.7	Interaction	1	1.7	0.231
						Error	8		
	SAO			22.8 \pm 0.9	28.9 \pm 1.3	Season	1	6.3	0.036
						Location	1	68.1	< 0.001
$\delta^{13}\text{C}$	Control	-14.1 \pm 1.3	-16.1 \pm 0.6	-10.9 \pm 0.2	-16.1 \pm 1.2	Interaction	1	15.2	0.054
						Error	8		
	SAO	-13.3 \pm 0.4	-12.5 \pm 0.3	-9.6 \pm 0.3	-12.0 \pm 0.8	Season	3	19.9	< 0.001
						Location	1	28.3	< 0.001
$\delta^{15}\text{N}$	Control	12.9 \pm 1.0	8.5 \pm 0.3	8.64 \pm 0.13	8.51 \pm 0.70	Interaction	3	3.2	0.033
						Error	37		
	SAO	16.5 \pm 0.5	15.5 \pm 0.3	15.5 \pm 0.3	14.9 \pm 1.1	Season	3	7.4	0.001
						Location	1	79.3	< 0.001
						Interaction	3	1.5	0.230
						Error	37		

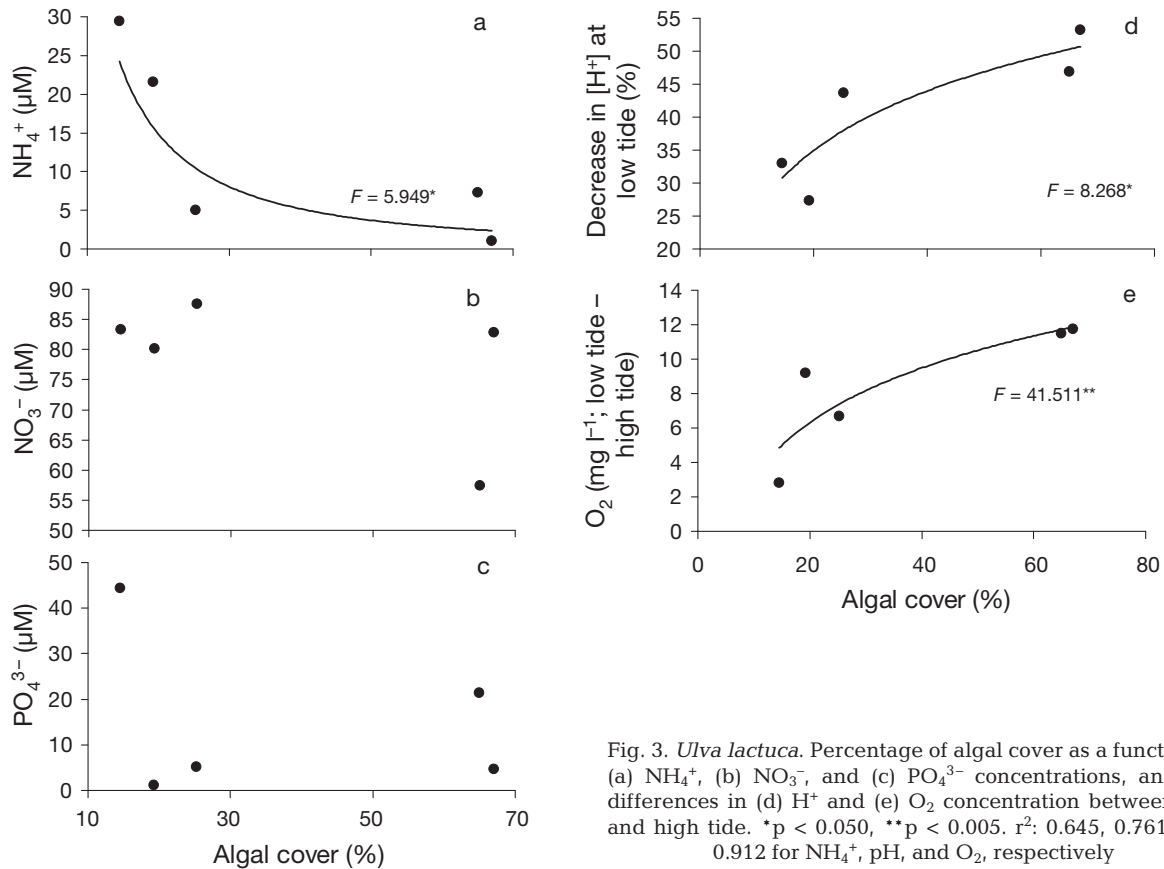


Fig. 3. *Ulva lactuca*. Percentage of algal cover as a function of (a) NH_4^+ , (b) NO_3^- , and (c) PO_4^{3-} concentrations, and the differences in (d) H^+ and (e) O_2 concentration between low and high tide. * $p < 0.050$, ** $p < 0.005$. r^2 : 0.645, 0.761, and 0.912 for NH_4^+ , pH, and O_2 , respectively

Relative effects of internal nutrient pools and herbivory on *Ulva lactuca* growth

Initial C, N, and P percentages and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were higher in *U. lactuca* thalli collected from the SAO than in those from the control channel (Table 1, values registered in fall). After 12 d of incubation in the SAO channel, there was no difference in the biomass accumulation rate of *U. lactuca* between low and high initial nutrient pool treatments but herbivory reduced the accumulation of *U. lactuca* biomass by 60.83% (Fig. 4, Table 2). Evidence of herbivory was the presence of holes as well as fragmentation of the margin in thalli incubated in the 10 mm mesh cages. There were no differences in the final percentages of C, N, and P between thalli with different initial nutrient contents but they were higher in thalli subjected to herbivory than in those not exposed to grazers (Fig. 4, Table 2). The same pattern was observed in the $\delta^{15}\text{N}$ signatures, while the $\delta^{13}\text{C}$ signatures were higher in thalli with high initial internal pool and in those subjected to herbivory. There were no differences between initial and final percentages of C, N, and P in thalli with high initial nutrient pools, while thalli with low initial internal pools showed an increase in nutrient contents up to values found in thalli with high ini-

tial internal pools (Fig. 4, Table 2). Thalli with lower initial internal pools had increased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures at the end of the experiment, whereas thalli with higher initial internal pools did not differ in their initial and final signatures (Fig. 4, Table 2). The percentage of algae consumed by grazers was also negatively related to both the N and C contents in thalli (Fig. 5a,b), positively related to $\delta^{13}\text{C}$ (Fig. 5d) and not related to the P content or to $\delta^{15}\text{N}$ (Fig. 5c,e).

Bottom-up vs. top-down effects on net growth of *Ulva lactuca*

There were no differences in the concentration of nutrients among cages with different mesh sizes of the control treatment and the ambient water ($F_{2,29} = 0.307$, $p = 0.820$; $F_{2,29} = 1.548$, $p = 0.215$; $F_{2,29} = 0.284$, $p = 0.836$ for NH_4^+ , NO_3^- , and PO_4^{3-} , respectively). Average concentrations of NO_3^- were 30.09% higher inside the cages with NO_3^- addition ($120.78 \pm 2.53 \mu\text{M}$ vs. $90.69 \pm 1.17 \mu\text{M}$, $F_{2,29} = 4.278$, $p = 0.042$) and there were no effects of mesh size ($F_{2,29} = 2.858$, $p = 0.083$). Phosphate showed interaction between mesh size and nutrient addition treatment ($F_{2,29} = 4.472$, $p = 0.014$), with PO_4^{3-} concentrations being 40 to 100% higher

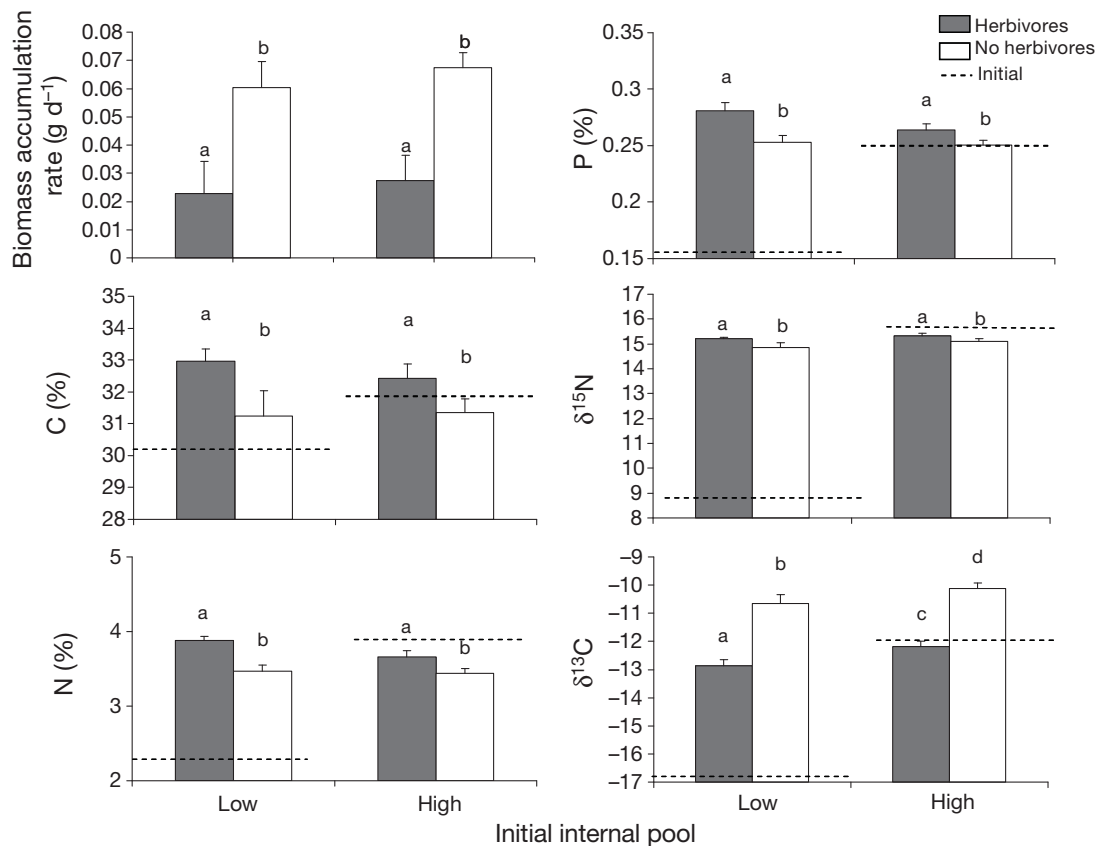


Fig. 4. *Ulva lactuca*. Biomass accumulation rate, C, N, and P percentages and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures in thalli with different initial nutrient pools and with (grey) or without (white) herbivory after 12 d of incubation in the San Antonio Oeste (SAO) channel. Different letters indicate significant differences (2-way ANOVA). Dashed horizontal lines indicate initial values before the incubation

inside cages with PO_4^{3-} addition and also higher in cages with 1 mm mesh ($7.67 \pm 0.98 \mu\text{M}$ vs. $5.35 \pm 0.51 \mu\text{M}$).

Herbivory reduced the biomass accumulation rate of *Ulva lactuca* by 59 and 55% in the control and PO_4^{3-} addition treatments, respectively (herbivory effect: $F_{1,29} = 17.503$, $p < 0.001$; Fig. 6). In contrast, the biomass accumulation rate of *U. lactuca* was not reduced by herbivory when NO_3^- was added. In the absence of herbivory, the addition of either NO_3^- or PO_4^{3-} did not lead to an increase in the *U. lactuca* biomass accumulation rate (effect of nutrients: $F_{2,29} = 0.122$, $p = 0.886$).

DISCUSSION

The results show that nutrients and biological products (e.g. photorespiration products and exudates) in San Antonio Bay are concentrated during low tide and diluted with every high tide. Despite the large flushing of water, nutrients remain in the tidal channels long enough to be assimilated by macroalgae, as was evi-

dent in the high internal nutrient pools and N isotopic signatures found in *Ulva lactuca* collected in the SAO channel. In addition, *U. lactuca* showed a high biomass accumulation rate but also supported an intense herbivory pressure that reduced the biomass accumulation by 60%. When nutrients were experimentally added, the accumulated biomass of *U. lactuca* was 45% higher with NO_3^- in the presence of herbivores, while there was no response in the accumulated biomass either with PO_4^{3-} addition or with NO_3^- in the absence of herbivores. Altogether, our results show that under macrotidal conditions, the relationship between bottom-up and top-down regulation may be different from that in other eutrophic systems subject to microtidal regimes where bottom-up forces clearly dominate the structure and dynamics of the ecosystem. In our study systems, water exchange during tidal changes can be large enough to diminish the potential negative effects of macroalgal accumulation (oxygen depletion, ammonium concentration) on herbivores such that herbivores can have a large impact on macroalgae. Below, we discuss the results of the different investigations in this study.

Table 2. Two-way ANOVA results comparing biomass accumulation rate, N, C, and P percentages, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of *Ulva lactuca* thalli from the San Antonio Oeste (SAO) and the control channels (initial internal pool factor) after 12 d of incubation in the SAO channel with or without small herbivores (herbivory factor)

	df	MS	F	p
Biomass accumulation rate				
Initial internal pool	1	0.001	0.374	0.545
Herbivory	1	0.012	16.163	<0.001
Interaction	1	0.001	0.015	0.904
%N				
Initial internal pool	1	0.125	2.569	0.120
Herbivory	1	0.816	16.712	<0.001
Interaction	1	0.073	1.505	0.230
%C				
Initial internal pool	1	0.380	0.140	0.708
Herbivory	1	15.500	5.770	0.023
Interaction	1	0.810	0.300	0.586
%P				
Initial internal pool	1	0.001	2.328	0.138
Herbivory	1	0.003	10.221	0.003
Interaction	1	0.001	1.345	0.256
$\delta^{15}\text{N}$ (‰)				
Initial internal pool	1	0.288	2.030	0.165
Herbivory	1	0.726	5.110	0.032
Interaction	1	0.036	0.260	0.617
$\delta^{13}\text{C}$ (‰)				
Initial internal pool	1	2.912	5.287	0.029
Herbivory	1	36.822	66.839	<0.001
Interaction	1	0.051	0.093	0.763

Links among water quality, internal nutrient pools, and macroalgal cover

Our results show that the concentrations of NH_4^+ , NO_3^- , and PO_4^{3-} were higher during low tide than during high tide and in the channel nearest to San Antonio Oeste. This suggests a main contribution of nutrients from land that is diluted with sea water during every high tide. The possible land-derived sources of nutrients in this system are a fish processing factory located on the shore in the innermost part of the SAO channel and septic systems from dwellings along the tidal channel. Salinity was not different between high and low tide. This indicates that the amount of freshwater input is relatively small, causing little change in salinity, or that evaporation may be high since the bay is located in a semi-desert ecosystem and a very large intertidal area is exposed during low tide. An estimation of the intertidal area exposed during low tide based on satellite images indicates that sea water during high tide covers an area of almost 120 km², with 81% of this area being exposed during low tide (J. P. Isacch pers. comm.).

The differences in values of water quality variables between the channels were also reflected in the internal nutrient pools as well as in the C and N stable isotopic signatures of *Ulva lactuca* thalli. There is evidence that macroalgae can assimilate the $\delta^{15}\text{N}$ signal of local land-derived N sources, which are higher due

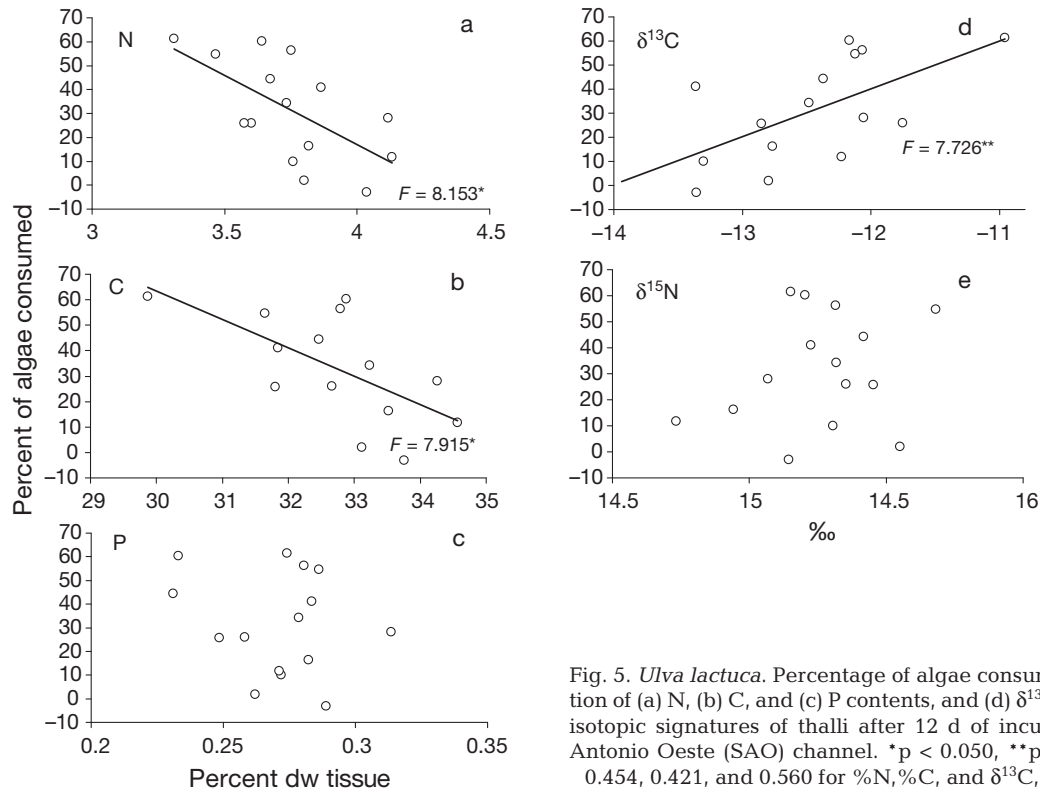


Fig. 5. *Ulva lactuca*. Percentage of algae consumed as a function of (a) N, (b) C, and (c) P contents, and (d) $\delta^{13}\text{C}$ and (e) $\delta^{15}\text{N}$ isotopic signatures of thalli after 12 d of incubation in San Antonio Oeste (SAO) channel. *p < 0.050, **p < 0.005). r² = 0.454, 0.421, and 0.560 for %N, %C, and $\delta^{13}\text{C}$, respectively

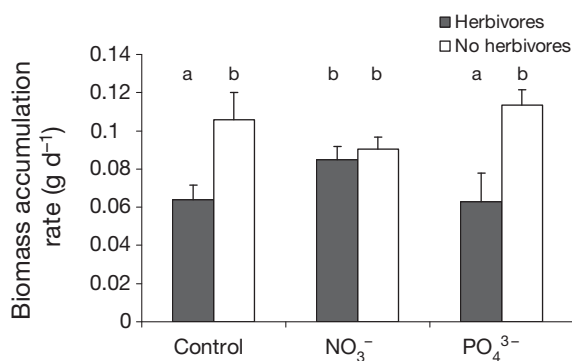


Fig. 6. *Ulva lactuca*. Biomass accumulation rate of thalli with NO₃⁻ and PO₄³⁻ addition and without nutrient addition (control), and with (black) and without (white) herbivory. Different letters indicate significant differences (2-way ANOVA)

to wastewater being the main source of N (McClelland & Valiela 1998, Martinetto et al. 2006, Oczkowski et al. 2008). In addition, $\delta^{13}\text{C}$ tends to be higher in eutrophic waters (Gearing et al. 1991, Oczkowski et al. 2008). The percentage of N and P in thalli of *U. lactuca* as well as in other macroalgal species increases with the availability of these elements in the environment (Pedersen & Borum 1996, Teichberg et al. 2007, 2008). Thus, the higher N and P contents and N and C stable isotopic signatures found in *U. lactuca* thalli in the SAO channel may be a result of the higher nutrient availability and the contribution of wastewater in this channel.

Nutrient concentrations reported in this study as well as in a previous study in the area (Martinetto et al. 2010) are high compared to values reported for US estuaries (Holmes 2008) and for other eutrophic coastal areas around the world (Teichberg et al. 2010). Concentrations of PO₄³⁻ in some sampling months were even higher than the highest values reported in these studies. Although N in wastewater is found mostly as NH₄⁺, DIN in the SAO channel was mostly NO₃⁻. This may be due to intense nitrification activity, in which nitrifying bacteria use energy contained in NH₄⁺, releasing NO₃⁻ as an end product (Valiela 1995). Moreover, there was a negative relationship between NH₄⁺ concentrations and macroalgal cover. This relationship could be due to macroalgal preference for NH₄⁺ as a N source (Pedersen & Borum 1997, Naldi & Wheeler 2002, Runcie et al. 2003, Cohen & Fong 2005). Although NH₄⁺ is the physiologically preferred form of N by macroalgae (Thomas & Harrison 1987), NO₃⁻-rich wastewater delivery to estuaries can also support blooms, and imprint the $\delta^{15}\text{N}$ signature of NO₃⁻ entering the estuary (McGlathery et al. 1997, McClelland & Valiela 1998, Cole et al. 2006).

pH and O₂ were also higher during low tide. During photosynthesis, many fast-growing green macroalgal

species raise the pH of the water near the surface of thalli as a result of uptake of HCO₃⁻ (Beer & Israel 1990, Larsson & Axelsson 1999). This involves extracellular dehydration of HCO₃⁻ by surface-bound carbonic anhydrase or direct uptake by anion exchange (Larsson et al. 1997). Under conditions of seawater salinity (>30) and pH (8.1 to 8.2), CO₂ concentrations are lower than HCO₃⁻ concentrations (0.014 and 2 mol m⁻³ respectively; Vymazal 1995). During intense photosynthesis, CO₂ concentration decreases with increased pH (Vymazal 1995, Oczkowski et al. 2008) near the algae. Elevated pH and O₂ concentration during low tide, therefore, may be a result of high photosynthetic activity. The positive relationship between changes in H⁺ and O₂ concentration and algal cover supports this idea.

Relative effects of internal nutrient pools and herbivory on *Ulva lactuca* growth

In the first experiment, the biomass accumulated by *U. lactuca* was reduced by herbivory by 60%. This reduction is quite high compared to the herbivory rates described in other eutrophic systems. For instance, in Maasholm Bay (Baltic Sea), the relative daily loss rates by herbivory were 29.3% in *Enteromorpha intestinalis* and 1.1% in *Pilayella littoralis* (Lotze & Worm 2000). In Waquoit Bay (MA), the grazing effect on *U. lactuca* in Childs River (a eutrophic estuary) was not significant (Morgan et al. 2003). San Antonio Bay seems to remain in an initial state of eutrophication where there is abundant food of good quality for herbivores. This is evident in the higher density of herbivores found in the SAO channel (Martinetto et al. 2010, present study). Based on the water quality values obtained, it seems probable that the system does not become anoxic (considering 4.6 mg O₂ l⁻¹ to be the lower limit for survival of most taxa (Vaquer-Sunyer & Duarte 2008)) despite the high nutrient availability due to the large water flushing during the tidal cycle. Thus, this could be a case in which eutrophication supports high densities of herbivores by increasing food availability and nutritional content rather than negatively affecting the survival of organisms.

This experiment also showed that there was no difference in the biomass accumulation rate between thalli with different initial internal nutrient pools. The time of incubation was long enough to allow the algae from the control channel to take up excess nutrients and increase internal pools. There are 2 possibilities that may support these results: (1) Nutrients were taken up and used for immediate growth at rates similar to algae from the SAO channel, or (2) the initial percentages of N in the thalli collected from the control

and the SAO channels (2.2 and 3.7% N in tissues, respectively) were above the value required for maximum growth (2.2% N of DW, Pedersen & Borum 1996). A previous study of *Ulva lactuca* from a low-nutrient estuary showed an increase in growth rate within 2 wk of nutrient addition (Teichberg et al. 2008), supporting the first scenario. Another study, however, showed a higher growth rate of *U. lactuca* in thalli with higher %N (Aguiar et al. 2003), concluding that algae growing in nutrient-poor waters grow slower even if they are transplanted into a nutrient-rich environment. Our results indicate that growth rate may depend on internal pool size up to some saturation threshold above which growth rate may remain constant at its maximum.

Percentages of C, N, and P were higher in thalli subjected to herbivory than in those not exposed to grazers. One possible explanation could be the excretion of N-rich compounds by grazers. For instance, Thomsen & McGlathery (2007) found higher growth rates and tissue N contents in *Gracilaria vermiculophylla*, *Ulva curvata*, and *Agardhiella subulata* subjected to herbivory and suggested that grazing could facilitate growth by algal uptake of N-rich compounds excreted by grazing snails.

There is evidence that the $\delta^{13}\text{C}$ in food web components and sediments from eutrophic estuaries are higher than in less productive waters (Gearing et al. 1991, Oczkowski et al. 2008). This pattern has been related to the uptake of HCO_3^- as a C source by macroalgae in nutrient-rich sea waters (Oczkowski et al. 2008). The $\delta^{13}\text{C}$ signature in HCO_3^- is $\sim 10\%$ higher than that in CO_2 (Zhang et al. 1995, Fry 2006). As we mentioned before, many marine macroalgal species can assimilate bicarbonate directly, so it is possible that the observed difference in the $\delta^{13}\text{C}$ signatures is due to differences in the uptake of HCO_3^- by macroalgae growing under different nutrient supply conditions. The $\delta^{13}\text{C}$ values in thalli from the control channel increased after incubation in the SAO channel, but this increment did not reach the values in thalli from the SAO channel. In contrast, the $\delta^{15}\text{N}$ after 12 d was the same for all the thalli independent of their origin. The turnover of N in *Ulva lactuca* is relatively rapid at 12 to 15 d (Aguiar et al. 2003). It is possible that the turnover of N was faster than that of C, allowing thalli from the control channel to reach the $\delta^{15}\text{N}$ value of *U. lactuca* from the SAO channel, but not the $\delta^{13}\text{C}$ value.

In addition, the percentage of algae consumed by grazers was negatively related to both N and C contents in the thalli and positively related to $\delta^{13}\text{C}$. Consumers select what they eat, and plant nutritional quality (often expressed as tissue N content) plays a key role in herbivore feeding patterns (Cruz-Rivera & Hay 2000, Giannotti & McGlathery 2001, Valentine & Heck 2001). Most studies show a positive relationship

between thalli N content and preference by herbivores (e.g. Zieman et al. 1984, McGlathery 1995, Giannotti & McGlathery 2001). In contrast, only a few studies show that herbivores can compensate for low food quality (in terms of N and C contents) by eating more (Cruz-Rivera & Hay 2000, Valentine & Heck 2001). In San Antonio Bay, the herbivores consumed more algae when the N and C contents in the thalli were lower, so it is probable that herbivores compensated for the differences in quality by increasing quantity. Thus, eutrophication may modify grazer–algae interaction by increasing food supply and quality (Korpinen et al. 2007). This modification may change life-history traits and population dynamics of grazers, and potentially affect the regulation of algal communities by herbivory.

Bottom-up vs. top-down effects on *Ulva lactuca* growth

In this system, the supply of N and P was so high that *U. lactuca* growth did not seem to be limited by these nutrients. This was evident in the lack of response in growth rate in the treatments with nutrient addition excluding grazers. In addition, the experiments show that herbivory plays an important role in controlling *U. lactuca* growth in San Antonio Bay. However, the results also show evidence that the effect of herbivory in controlling *U. lactuca* growth could be broader and directly or indirectly influences the response of algae to an increase in N supply. Top-down processes have been shown to affect bottom-up processes in coastal marine systems (Porter 1976, Sterner 1986, Bracken & Stachowicz 2007). For instance, invertebrates may excrete inorganic N (mainly NH_4^+), enhancing the diversity of seaweeds (Williamson & Rees 1994, Bracken & Nielsen 2004). Herbivores may also reduce N uptake by selective grazing on specialized tissues (Bracken & Stachowicz 2007). In terrestrial ecosystems, herbivores may stimulate N uptake in grasses by removing physiologically less active leaves, inducing specific enzyme activities, and increasing stomatal conductance among others mechanisms (see Anderson et al. 2006). However, our first experiment showed a negative relationship between the percentage of algae consumed and the N content in thalli, suggesting compensatory feeding. It is possible that when N was added, herbivores did not consume enough tissue to reduce algal growth, resulting in similar growth rates of *U. lactuca* with and without herbivory. Lapointe & Tenore (1981), in an experimental outdoor study, reported an increase in the growth rate of *Ulva fasciata* when NO_3^- was added under low light condition. Light attenuation inside the incubation cages used in our experiments was tested and no difference was found

between the inside and outside of cages (Teichberg 2007). Given the similarity between the 1 and 10 mm mesh size cages, we assume that there is no difference in light attenuation between cages; hence, it seems unlikely that the observed difference in growth rates between cages with different mesh sizes when NO_3^- was added was a cage artifact due to light attenuation. To our knowledge, this is the first evidence showing that the combined presence of herbivores and increase in N supply may stimulate the accumulation of green macroalgal biomass in a system functioning with unusually high nutrient availability and herbivory. Under these conditions, top-down and bottom-up forces interact in the regulation of macroalgal proliferation. Although the ultimate mechanism operating is unknown, the high herbivory pressure and the response to NO_3^- addition only when herbivores were present provide evidence that both regulatory forces act conjointly.

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