

# Impact of burrowing crabs on C and N sources, control, and transformations in sediments and food webs of SW Atlantic estuaries

Florencia Botto<sup>1,\*</sup>, Ivan Valiela<sup>2</sup>, Oscar Iribarne<sup>1</sup>, Paulina Martinetto<sup>1,2</sup>, Juan Alberti<sup>1</sup>

<sup>1</sup>Departamento de Biología, Universidad Nacional de Mar del Plata, CC 573 Correo Central B7600WAG, Mar del Plata, Argentina

<sup>2</sup>Boston University Marine Program, Marine Biological Laboratory, 7 MBL St. Woods Hole, Massachusetts 02543, USA

**ABSTRACT:** The intertidal burrowing crab *Chasmagnathus granulatus* is the dominant species in soft bare sediments and vegetated intertidal areas along the SW Atlantic estuaries (southern Brazil, 28° S, to northern Patagonia, 42° S). *C. granulatus* creates burrows that can reach densities of 60 burrows m<sup>-2</sup>, and its burrowing activities increase water and organic matter content of sediments. To evaluate the long-term effect of burrows on the origin and transformation of accumulated organic matter within sediments, we compared C and N stable isotope signatures of sediments, plants, and consumers within areas with and without crabs. <sup>15</sup>N signatures of sediments and primary producers were enriched by 3 to 7‰ in areas with crabs. The enrichment was present in 4 different Argentine estuarine environments (Mar Chiquita coastal lagoon, 37° 46' S, 57° 19' W, Bahía Blanca, 38° 50' S, 62° 07' W, San Blas, 40° 33' S, 62° 14' W, San Antonio, 40° 48' S, 64° 52' W). Enrichment owing to crab activity appeared to overwhelm possible different N loads, anthropogenic influence, and other properties. Crab activity thus uncoupled the nitrogen dynamics in sediments from external controls. Enrichment of the heavier isotope of N could be the result of an increase in denitrification rates in areas with burrows. Crabs therefore forced faster transformation of available to unavailable nitrogen, making less inorganic nitrogen available to deeper waters. Food webs in areas with and without crabs were similar in shape, but less mobile benthic organisms (nematodes, fiddler crabs and the polychaete *Laeonereis acuta*) showed enriched N isotopic signatures. The benthic food web seemed separate from that of suspension feeders or water column consumers. Benthic microalgae were an important source for infauna, and marsh plants were particularly important for burrowing crabs.

**KEY WORDS:** Estuaries · Food webs · Stable isotopes

Resale or republication not permitted without written consent of the publisher

## INTRODUCTION

An important issue in estuarine ecology is to identify the relative importance of different primary producers supporting estuarine food webs. In some salt marshes, *Spartina* species play a fundamental role (Currin et al. 1995); in others, benthic microalgae (Sullivan & Moncreiff 1990) or macroalgae and phytoplankton (Deegan & Garritt 1997) are more important. The relative area occupied by salt marshes, bathymetry, and tidal amplitude (Kwak & Zedler 1997) and land cover types on watersheds (McClelland et al. 1997, Valiela et al. 1997,

McClelland & Valiela 1998) may also affect the relative contribution by different producers.

Burrowing species, including shrimps and crabs, usually dominate the intertidal of estuarine environments. These organisms construct extensive feeding galleries in the soft-sediment benthos affecting habitat complexity in soft bottoms (Peterson 1977, 1979, Bird 1982). Burrowing species may structure the community by feeding directly on other organisms (Botto & Iribarne 2000, Daleo et al. 2003) or plants (Bortolus & Iribarne 1999), or by competing for space or food (Posey 1990). Sediment disturbance by burrowers can

\*Email: fbotto@mdp.edu.ar

affect a variety of population (Rowden et al. 1998, Botto & Iribarne 1999) and ecosystem processes (Rhoads & Young 1970, Rice & Rhoads 1989, Ziebis et al. 1996).

One of the most important bioturbators of SW Atlantic estuaries is the burrowing crab *Chasmagnathus granulatus* (Iribarne et al. 1997), whose burrows can reach densities of up to 60 m<sup>-2</sup>, and assemblages can occupy 80% of the intertidal areas (Fig. 1). These crabs inhabit the intertidal in both the soft bare sediment flats and the lower salt marsh zones composed of *Spartina densiflora* (Iribarne et al. 1997), *S. alterniflora*, and *Sarcocornia perennis* (formerly *Salicornia ambigua*). In bare sediments, burrows are funnel-shaped, have large entrances (up to 14 cm in diameter), and extend up to 0.4 m into the sediment (Botto & Iribarne 2000). In vegetated areas, burrows

are cylindrical and deeper (up to 1 m). *C. granulatus* affects species composition and abundance of infauna (Botto & Iribarne 1999), marsh grass production, and soil quality (Iribarne et al. 1997, Bortolus & Iribarne 1999). Burrows that are funnel-shaped accumulate sediments and contain enhanced organic matter content (measured as ash-free dry weight, Botto & Iribarne 2000) and abundant meiofaunal organisms (Iribarne et al. 1997). Burrows of crabs may therefore alter food resources available to consumers and associated nutrient dynamics.

Stable-isotope signatures of C and N help to understand the origin and pathway of organic matter in food webs (Peterson et al. 1985, Michener & Schell 1994). Different producers may bear recognizably different  $\delta^{13}\text{C}$  signatures (Lajtha & Michener 1994). In addition, when stable isotopes are metabolized by organisms, there is some degree of fractionation since isotopes of different weights do not participate equally in reactions. For example, there is a fractionation of about 3 to 4‰ for N isotopes, and about 1.5‰ for C isotopes, at every trophic step (Michener & Schell 1994). This isotopic fractionation can provide information on the position of species within the trophic web.

Differences among sites in N isotopic signatures of producers can be the result of different external anthropogenic pollution (McClelland & Valiela 1998), animal waste (Kendall 1998), or fertilizers (Macko & Ostrom 1994) on N sources. N isotope signatures not only depend on the external origin of the N, but are also affected by internal biogeochemical transformations, such as N-fixation, nitrification, or denitrification (Kendall 1998). NO<sub>3</sub> produced by nitrification has <sup>15</sup>N-depleted signatures and the isotopic ratio of the remaining NO<sub>3</sub> increases after denitrification (Kendall 1998). N uptake by plants in soils only causes small fractionation and therefore, isotopic composition of plants, in most cases, reflects that of the soil organic matter (Kendall 1998).

In this paper, we use isotopic values of N and C in plants, consumers, and sediments in areas with and without crabs of the Mar Chiquita coastal lagoon (37° 46' S, 57° 19' W; Fig. 2), to evaluate the relative influence of burrowing crabs on habitat and food web. This coastal lagoon is a body of brackish water (salinity range: 6 to 33‰) affected



Fig 1. Photograph of areas with burrows. (A) Bare mudflat with burrows in San Blas. (B) Burrowing area in *Spartina densiflora* marsh. (C) Close-up of a burrowing crab *Chasmagnathus granulatus*. Photo credits: O. Iribarne (A,B), P. Ribeiro (C)

by tides of  $\leq 1$  m and with extensive mudflats surrounded by areas of mostly *Spartina densiflora* cordgrass (Bortolus & Iribarne 1999). We also used isotopic ratios of N and C in plants to evaluate the effect of burrowing crabs in another 3 estuaries: Bahia Blanca ( $38^{\circ} 50' S$ ,  $62^{\circ} 07' W$ ), San Blas ( $40^{\circ} 33' S$ ,  $62^{\circ} 14' W$ ), and San Antonio ( $40^{\circ} 48' S$ ,  $64^{\circ} 52' W$ ; Fig. 2). Bahia Blanca is one of the largest SW Atlantic estuarine environments, with a macrotidal regime (up to 4 m) and is close to an important industrial city (Bahia Blanca city, approximately 500 000 inhabitants). San Blas is a pristine estuarine environment near a small tourist village (<500 people) with 2.5 m of tidal amplitude. San Antonio bay is an estuary with tides of up to 9.3 m with a nearby town of 15 000 inhabitants. These estuaries, all inhabited by high densities of crabs, allowed us to compare their effect on estuaries with different sources of organic matter and different influences of anthropogenic activity.



Fig. 2. Map showing the location of the different sampling sites

## MATERIALS AND METHODS

The effects of burrowing crabs on C and N isotope values in sediments and in the food web were evaluated within areas with and without crab burrows in the Mar Chiquita lagoon. For all samplings, 3 intertidal areas with burrows and 3 without burrows were selected. Areas described as 'with burrows' were areas with a mean density of more than 20 burrows  $m^{-2}$  and with an extension of more than 1 ha (see Fig. 1 as example). The areas were similar in bathymetry, salinity, slope of the mudflat and tidal amplitude.

Samples of sediments were randomly taken from each area and inside burrows. Sediment was taken at 3 depths: (1) 0 to 1 cm, (2) 5 to 5.1 cm, and (3) 10 to 10.1 cm. Samples of the sediment accumulated in burrows were extracted by inserting a tube and collecting the material of the first centimeter of the end of the burrow. The percentages of C and N, and their isotopic ratios were evaluated in sediments comparing areas with and without crabs. Samples of sediments were exposed to HCl to extract any carbonate present. Samples were dried at  $70^{\circ}C$ , ground to fine powder, and weighed and loaded into tin capsules. 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  $\delta$  values as ‰:  $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $X = {}^{13}C$  or  ${}^{15}N$ , and  $R$  is the corresponding ratio  ${}^{13}C: {}^{12}C$  or  ${}^{15}N: {}^{14}N$ . Standards used were Vienna Pee Dee Belemnite for C, and  $N_2$  for N. Differences in the percentages of N and C, and C:N ratios between areas with and without burrows and the 3 depth layers were evaluated with 2-fixed factors ANOVA (Zar 1999). Differences in  $\delta^{15}N$  and  $\delta^{13}C$  among surface sediments of burrow and non-burrow areas, and the sediment accumulated inside burrows were evaluated with ANOVAs (Zar 1999).

To evaluate differences in isotopic ratios on the food web of areas with and without crabs, we randomly sampled specimens of the major producers and consumers and measured their  $\delta^{13}C$  and  $\delta^{15}N$ . Samples of the above-ground part of *Spartina densiflora* were dried and ground. Samples of benthic microalgae were extracted from sediment samples (10 cm diameter, 2 cm deep) following Couch (1989). This method was based on algal migration to a light source. The Couch method is designed to permit collection, avoiding contamination with detritus. Before isotopic analysis, we examined the samples microscopically to ensure they were free of detritus. Benthic consumers were collected for isotopic analysis including 3 crab species: *Chasmagnathus granulatus* (adults and juveniles), *Cyrtograpsus angulatus*, and *Uca uruguayensis*; the razor clam *Tagelus plebeius*; and the 2 polychaetes *Laeonereis acuta* and *Neanthes succinea* (Botto et al.

1998, 2000, Botto & Iribarne 1999). Individuals of *C. granulatus* were collected from the marsh habitat and bare sediment. The crabs and the razor clams were extracted by hand or digging. Polychaetes were extracted from sediment samples (10 cm diameter, 20 cm deep) by sieving through a 0.5 mm mesh screen. All organisms were kept in estuarine-filtered water for 4 h to empty gut content (to measure only C and N assimilated). Shells of clams were not included in the analysis. Samples of the main juvenile fish species were collected using a beach seine (1 cm mesh size) towed for 50 m, parallel to the shore. To prevent unassimilated food from affecting the measurement of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , only the white muscle of fishes was used. Composites of at least 3 individuals of each species were made to make samples more representative. Both animals and plants were rinsed with deionized water, dried at 60°C for 2 to 3 d, and prepared for isotope analysis as described before.

We also obtained samples of water from areas with and without burrows. From this water, we obtained organisms from the water column retained with plastic plankton nets of 100  $\mu\text{m}$  mesh size after discarding the coarser material with a mesh size of 250  $\mu\text{m}$ . We also obtained a portion of material finer than 100  $\mu\text{m}$ , mainly composed of phytoplankton. Both kinds of samples were filtered with Whatman G/F fiberglass filters (previously ashed at 500°C) in a low-pressure vacuum.

To test whether differences in N isotope signatures differed for one area or another, or were associated with the grasses themselves, we transplanted *Spartina densiflora* from areas with burrows to areas without burrows, from areas without burrows to areas with burrows, and controls of plants extracted from each area (with or without burrows) and planted again in the same area. All transplants were performed extracting a core of 10 cm diameter and 35 cm depth with the surrounding sediment and deployed again. After 45 d, a sample was taken of the new growth to estimate  $\delta^{15}\text{N}$  values as described above.

To ascertain whether the relationship found in Mar Chiquita could be extended to other estuaries in the SW Atlantic, we also sampled areas with and without burrows in Bahia Blanca, San Blas, and San Antonio. Samples of the most abundant marsh species in areas inhabited by crabs from each estuary were taken. *Spartina densiflora* and *Sarcocornia perennis* were collected at the Bahia Blanca estuary; *S. densiflora*, *S. alterniflora*, and *S. perennis* were collected at San Blas; and samples of *Spartina alterniflora* and *S. perennis* were obtained from San Antonio. Samples of the above-ground parts of plants were extracted by hand from areas with and without burrows. Areas with and without crabs were selected as described for Mar Chiquita.

Differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values between areas for each species and each estuary were compared between areas with and without crabs with *t*-tests after correcting for non-homogenous variances as necessary (Zar 1999).

## RESULTS

The sediment in the presence of burrowing crabs had double the %N content (Table 1) and a higher %C content than sediment with no burrowing crabs (Table 1). Surface sediments seemed to collect in burrows, as made evident by the similarity of values of %N and %C (Table 1).

$\delta^{15}\text{N}$  values were about 2 to 4‰ higher in sediment where crabs had made burrows. There were no significant difference in  $\delta^{13}\text{C}$  (Table 2, Fig. 3). Sediment isotopic ratios inside burrows were similar to those in bulk sediment (Fig. 3). There were no differences in stable isotopes with depth in either type of sediment for  $\delta^{13}\text{C}$ , but  $\delta^{15}\text{N}$  values were lower in the deepest layer (Fig. 3, Table 2).

The 3 primary sources of organic matter in the Mar Chiquita coastal lagoon are *Spartina densiflora*, benthic microalgae, and phytoplankton. These primary producers did not differ in  $\delta^{13}\text{C}$  isotopes (Table 3, Fig. 4) between areas with and without burrows.  $\delta^{15}\text{N}$  values of microalgae and *S. densiflora*, however, were higher in areas with burrows than in areas without burrows (Table 3, Fig. 4).

Table 1. Percentages of N and C, and C:N ratios of sediment from inside burrows and surface sediment of areas with and without burrows. \* $p < 0.05$

	%N	%C	C/N
<b>With burrows</b>			
Inside	0.08 ± 0.02	1.4 ± 0.35	18.1 ± 2.7
Surface	0.09 ± 0.02	1.4 ± 0.44	14.8 ± 3.7
<b>Without burrows</b>			
Surface	0.04 ± 0.02	0.9 ± 0.6	24.3 ± 8.5
$F_{(2,15)}$	16.6*	1.8	4*

Table 2. ANOVA results testing the effects of area (with and without crabs) and depth (3 layers) on C and N isotope ratios. \* $p < 0.05$

Source of variation	df	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
		MS	F	MS	F
Area	1	26.4	11.7*	2.3	2.8
Depth	2	18.5	8.2*	2.5	3.2
Area × Depth	2	2.4	1.07	0.3	0.3
Error	12	2.25	0.8		

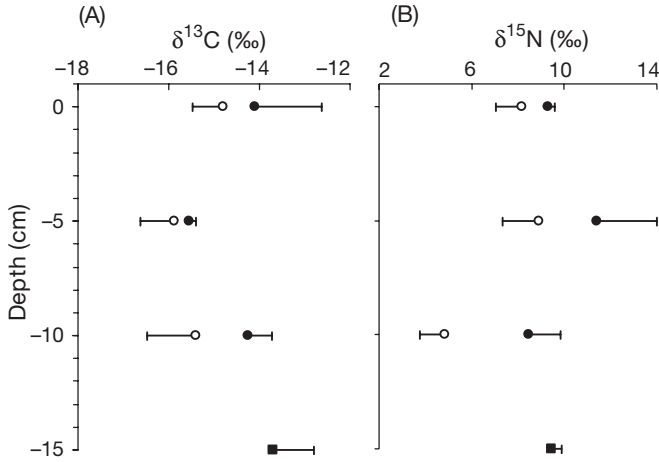


Fig. 3. (A) C and (B) N stable-isotope ratios (mean  $\pm$  1 SE) of sediment at 3 depths in burrow areas (●) and areas without burrows (○) and of sediment from inside burrows (■)

Consumers showed variable values of both C and N stable isotopes.  $\delta^{15}\text{N}$  values of deposit feeders (the polychaete *Laeonereis acuta*, the fiddler crab *Uca uruguayensis*, were higher in areas with burrows (Table 3, Fig. 4). Although the absence of replicate samples did not allow statistical comparisons for nematodes, the aggregated sample of 1000 nematodes also showed a N value 2.26‰ higher in areas with crabs. No differences in  $\delta^{15}\text{N}$  values were found for the other polychaete species, the razor clams, the other crabs, or

fish species (Table 3, Fig. 4). C isotope ratios showed no differences in any species.

All plants from areas with crabs in Mar Chiquita, Bahia Blanca, San Blas, and San Antonio showed higher  $\delta^{15}\text{N}$  than those from areas without crabs (Fig. 5). The difference persisted among the macrophyte species, even though there was significant variation in signatures from one species to another (Fig. 5).

$\delta^{15}\text{N}$  values of *Spartina densiflora* changed when transplanted from different areas.  $\delta^{15}\text{N}$  of plants transplanted from areas without burrows to areas with burrows increased from 3.3 to 6.9‰ ( $t = 10.6$ ,  $df = 4$ ,  $p < 0.05$ ).  $\delta^{15}\text{N}$  decreased from 8.2‰ in areas with burrows to 5.4‰ when transplanted to areas without burrows ( $t = 6.6$ ,  $df = 4$ ,  $p < 0.05$ , Table 4).

Different types of producers supplied the fixed C that moved up the food webs in areas with and without crabs. Examination of the position of consumers along the x-axis of Fig. 6 suggested that the 3 primary sources of organic matter in the Mar Chiquita coastal lagoon (*Spartina densiflora*, benthic microalgae, and phytoplankton) showed differences in C ratios, and that all of them contributed to the food webs. High values (less negative) of  $\delta^{13}\text{C}$  associated with *S. densiflora* were found for the fiddler crab *Uca uruguayensis* in crab and non-crab areas. The other crab species *Cyrtograpsus angulatus* and the razor clam and all fishes were more depleted in  $^{13}\text{C}$ , indicating mixed diets of phytoplankton and benthic microalgae. The major food for nematodes and the

Table 3.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean  $\pm$  SE) of primary producers and consumers in areas with and without crab burrows of the Mar Chiquita coastal lagoon. C: crustacean; B: bivalve; P: polychaetes; F: fish. \* $p < 0.05$

		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
		With burrows	Without burrows	With burrows	Without burrows
<b>Primary producers</b>					
<i>Spartina densiflora</i>		10.6 $\pm$ 0.6	1.8 $\pm$ 0.8*	-13.1 $\pm$ 0.0	-13.3 $\pm$ 0.04
Benthic microalgae		6.9 $\pm$ 0.4	4.9 $\pm$ 0.2*	-16.1 $\pm$ 0.4	-16.3 $\pm$ 0.2
Phytoplankton		8.7 $\pm$ 0.4	8.7 $\pm$ 0.4	-20.2 $\pm$ 0.4	-20.4 $\pm$ 0.4
<b>Deposit feeders</b>					
Nematodes		8.8	6.5	-14.8	-15
<i>Laeonereis acuta</i>	P	12.2 $\pm$ 0.04	10.9 $\pm$ 0.1*	-15.5 $\pm$ 0.1	-15.8 $\pm$ 0.1
<i>Uca uruguayensis</i>	C	11.8 $\pm$ 0.1	8.4 $\pm$ 0.2*	-12.7 $\pm$ 0.2	-12.9 $\pm$ 0.2
<b>Suspension feeder</b>					
<i>Tagelus plebeius</i>	B	11.9 $\pm$ 0.2	12.1 $\pm$ 0.1	-18.3 $\pm$ 0.2	-18.3 $\pm$ 0.1
<b>Predators — omnivores</b>					
<i>Neanthes succinea</i>	P	12.6 $\pm$ 0.04	13.4	-17.4 $\pm$ 0.2	-15.2
<i>Cyrtograpsus angulatus</i>	C	9.9 $\pm$ 0.1	10.1 $\pm$ 0.1	-18.4 $\pm$ 0.3	-18.7 $\pm$ 0.5
<i>Brevoortia aurea</i>	F	14.1 $\pm$ 0.3	12.9 $\pm$ 0.4	-17.6 $\pm$ 0.3	-17.1 $\pm$ 0.1
<i>Micropogonias furnieri</i>	F	15.6 $\pm$ 0.2	15.03 $\pm$ 0.1	-15.8 $\pm$ 0.3	-16.3 $\pm$ 0.2
<i>Odontesthes argentiniensis</i>	F	17.7 $\pm$ 0.3	15.8 $\pm$ 0.2	-17 $\pm$ 0.1	-17.2 $\pm$ 0.1
<i>Paralichthys orbygnianus</i>	F	14.5	13.6	-15.6	-16.6
<i>Ramnogaster arcuata</i>	F	15.3	13.9 $\pm$ 0.3	-17.7 $\pm$ 0.5	-17.5
<b>Zooplankton</b>		11.3 $\pm$ 0.6	11.5 $\pm$ 0.6	-19.1 $\pm$ 0.4	-20 $\pm$ 0.5

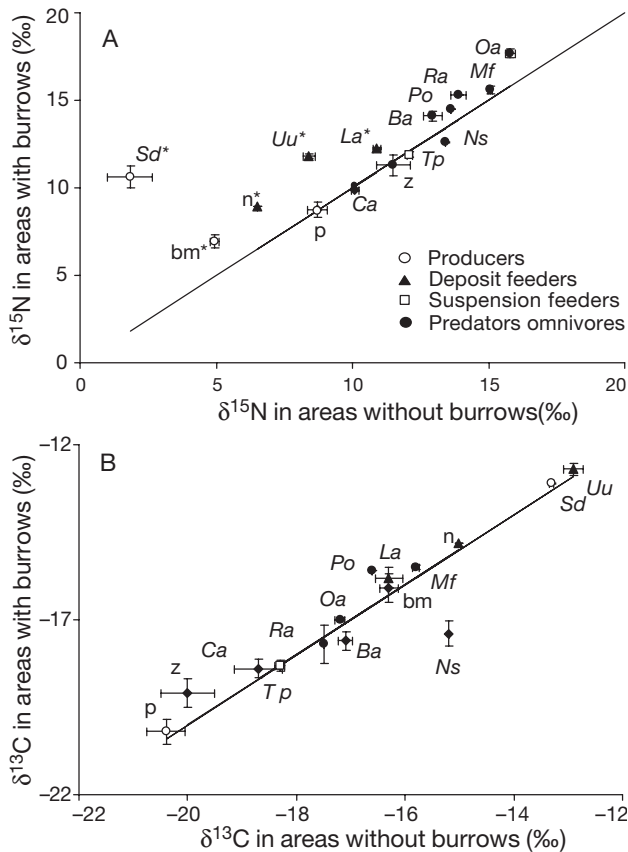


Fig. 4. Isotope (A) N and (B) C values (mean  $\pm$  1 SE) from areas with burrows versus areas without burrows at Mar Chiquita for *Spartina densiflora* (Sd), benthic microalgae (bm), phytoplankton (p), zooplankton (z) the crab *Cyrtograpsus angulatus* (Ca), the polychaete *Laeonereis acuta* (La), the polychaete *Neanthes succinea* (Ns), the razor clam *Tagelus plebeius* (Tp), nematodes (n), the fiddler crab *Uca uruguayensis* (Uu), the fishes *Brevoortia aurea* (Ba), *Ramnogaster arcuata* (Ra), *Paralichthys orbygnianus* (Po), *Micropogonias furnieri* (Mf), and *Odontesthes argentinensis* (Oa)

polychaete *Laeonereis acuta* seemed to be benthic microalgae.

The  $\delta^{15}\text{N}$  found in the food webs betrayed trophic level position. N values of consumers ranged between 7 and 17‰. Nematodes and *Chasmagnathus granulatus* were low in the food web, while the fishes *Odontesthes argentinensis* and *Ramnogaster arcuata* were in the top trophic level. The presence or absence of crabs did not change the trophic positions of the species except for the polychaete *Neanthes succinea*, which showed a shift in diet with enriched  $^{13}\text{C}$  in areas without burrows. The more evident difference between areas with and without burrows was the presence of *C. granulatus*.  $\delta^{13}\text{C}$  of both juveniles and adults of *C. granulatus* showed values similar to *Spartina densiflora*, but the N ratios were between values of *S. densiflora* from areas with and without burrows.

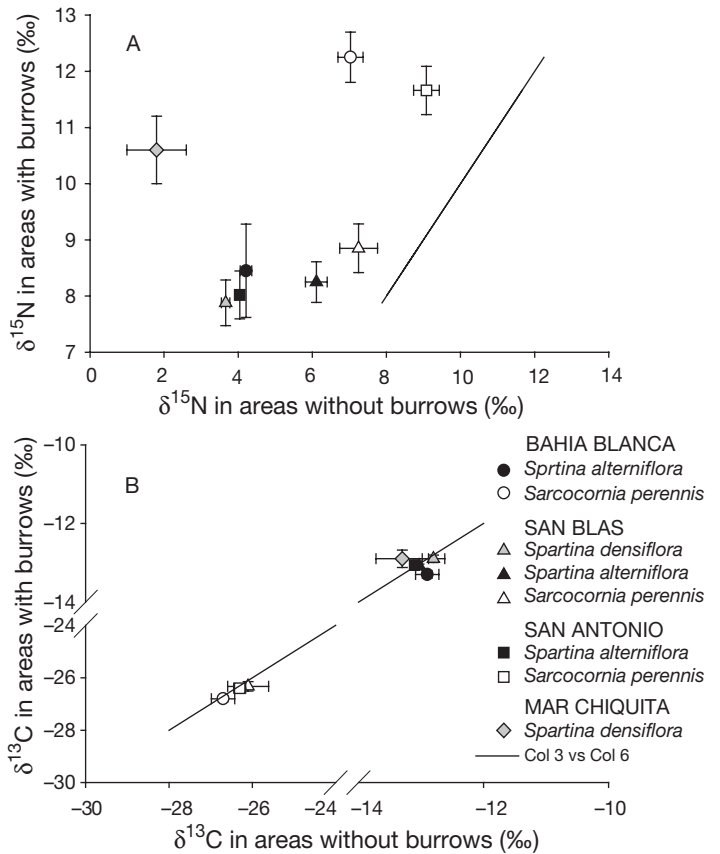


Fig. 5. Isotope N (A) and C (B) values (mean  $\pm$  1 SE) from areas with burrows versus areas without burrows for different plant species from the 4 estuaries studied

DISCUSSION

*Chasmagnathus granulatus* affected sediments by increasing organic matter content (Botto & Iribarne 2000), particularly accumulating N and decreasing the C:N ratio. Botto & Iribarne (2000) showed that although these crabs could be actively selecting to be present in areas with enhanced organic matter, they themselves increased organic matter accumulation through the passive deposition of detritus in burrows. The relative increase of N in crab beds agrees with the

Table 4.  $\delta^{15}\text{N}$  of *Spartina* shoots collected from areas with and without burrows, before being transplanted (Untransplanted), in plants dug out and replanted in the same area (Controls), and in transplanted plants to areas with and without burrows (transplanted)

	With burrows	Without burrows
Untransplanted	10.6 $\pm$ 0.6	1.8 $\pm$ 0.8
Control	8.2 $\pm$ 0.05	3.3 $\pm$ 0.17
Transplanted	6.9 $\pm$ 0.29	5.4 $\pm$ 0.43

better feeding conditions of polychaetes (Palomo et al. 2004) living in those areas.

The origin of C accumulated in sediment was not affected by burrowing crabs, as indicated by values of  $\delta^{13}\text{C}$  that showed no differences between areas with and without burrows.  $\delta^{15}\text{N}$  values of sediments were always higher in areas with burrows. Both kinds of sites (with and without crabs) are spatially very close (in some cases less than 100 m), so it seems unlikely to expect differences in N sources among areas. It seems more likely that local biogeochemical processes in burrowing areas may be responsible for changing N isotopic signatures. Denitrification increases the N isotopic signatures (Kendall 1998), and may be stimulated by crab burrows, which increase the oxic–anoxic interface. In anoxic sediments of marshes, there may be 60 burrows  $\text{m}^{-2}$ . Considering mean entrance diameters (3.6 cm) and depth (41 cm), and assuming straight tube shapes (Iribarne et al. 1997), burrows may increase the surface area by, on average, 3.7 times. This increase in the area of the aerobic–anaerobic interface can increase nitrification–denitrification coupling, given that the nitrate produced by nitrification in aerobic conditions is removed by denitrification in anaerobic conditions. Increasing nitrate production in anaerobic estuarine sediments (Jenkins & Kemp 1984) may increase denitrification rates. Burrowing areas can occupy up to 80% of intertidal zones in estuaries of the SW Atlantic. An increase in denitrification rates in burrow areas may decrease the supply of nitrates to the water column. Sediments with burrows also retain detritus, thus lowering transport of organic matter down-estuary. The effects of burrowing organisms could therefore have important ecological implications for preventing eutrophication in estuarine systems. Burrowing species, such as the crabs included here, may have strong top-down effects through their activity affecting survival, growth, or recruitment of other benthic fauna (Botto & Iribarne 1999). Our results suggest that these species can also influence bottom-up effects by affecting nutrient dynamics.

The uptake of N by primary producers reflected the differences of N-signatures of sediments, which were also enriched in areas with the presence of burrows. When plants were transplanted from one area to the other, new shoots also changed isotope values. After 45 d, values had not reached the value of controls. Given that plants were transplanted with their original surrounding sediment, this time period was probably not long enough to completely change isotope values. The results of this experiment indicate that differences in N isotopes between areas with and without burrows were not due to intrinsic factors of the plants, but were rather the product of changes in the isotope signature of nutrients in sediments from those areas.

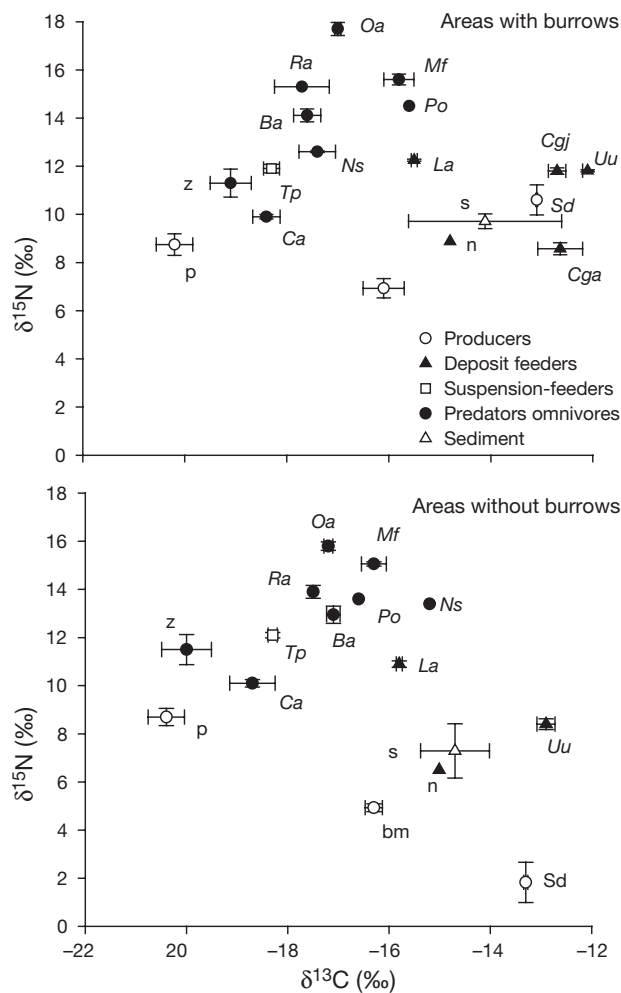


Fig. 6.  $\delta^{15}\text{N}$  versus  $\delta^{13}\text{C}$  (mean  $\pm$  1 SE) for the complete food web for burrow and non-burrow areas. *Spartina densiflora* (Sd), benthic microalgae (bm), phytoplankton (p), zooplankton (z), the crab *Cyrtograpsus angulatus* (Ca), the polychaete *Laeonereis acuta* (La), the polychaete *Neanthes succinea* (Ns), the razor clam *Tagelus plebeius* (Tp), nematodes (n), the fiddler crab *Uca uruguayensis* (Uu), and the fishes *Brevoortia aurea* (Ba), *Ramnogaster arcuata* (Ra), *Paralichthys orbygnianus* (Po), *Micropogonias furnieri* (Mf), and *Odontesthes argentinensis* (Oa)

Even though there were differences in anthropogenic wastewater N input among the 3 estuarine environments studied, the major feature associated with within-estuary difference in  $\delta^{15}\text{N}$  values was the presence or absence of crabs. Recent studies have shown strong variability of plants between nearby areas (Cloern et al. 2002) and, consequently, the applicability of N-isotope signatures to trace the linkage between producers and their consumers was questioned. What is important is to determine the factors that are producing this variability. Our data show that biological factors such as the presence of burrowing organisms must be considered.

The consumers that reflected the differences of primary producers were nematodes, the polychaete *Laeonereis acuta*, and the fiddler crab *Uca uruguayensis*. Fishes are predators, and did not show differences between sites although some species use areas with burrows more frequently (Martinetto 2001). This is because their muscle signature averages signatures of food taken over home ranges that likely include both types of areas. The razor clam is a suspension feeder and did not show differences among sites. Processes affecting isotopic signatures of organic matter in sediments can therefore produce a cascade effect on consumers associated with the sedimentary environment, particularly deposit feeders.

The ecological significance of the variation in isotopic signatures among individuals within species was recently highlighted for different systems. In freshwater systems, higher variability was found in less mobile consumers, which could be reflecting high among-patch variation in resources (Lancaster & Waldron 2001). In fish reef assemblages, variability in N signatures was related to differences in fish size related to differences in trophic levels of individuals of a population (Jennings et al. 1997). Our study shows that without considering the presence of burrows, differences in N isotopic ratios would be incorporated in intra-population variability, complicating the identification of trophic pathways.

The 3 sources of organic matter in the Mar Chiquita coastal lagoon (salt marsh, benthic microalgae and phytoplankton) showed distinct  $\delta^{13}\text{C}$  values, providing a reasonable method to evaluate sources of energy for the food web in this system.  $\delta^{13}\text{C}$  values of sediment (between  $-12.6$  and  $-15.5\text{‰}$ ) suggest a strong influence of *Spartina* marshes and benthic microalgae in the accumulation of organic matter in intertidal sediments of the Mar Chiquita coastal lagoon. The C isotopic signature of consumers showed that the 3 sources of organic matter were important to support the food web. The 2 burrowing crab species (*Chasmagnathus granulatus* and *Uca uruguayensis*) are considered deposit feeders and their C isotopic signature showed the importance of *Spartina* debris as a food source. The N ratio of crabs was, however, lighter than expected for 1 trophic level fractionation in areas with burrows, probably because the origin of detritus accumulated in those areas is supplied from both areas with and without burrows. Razor clams and the crab *Cyrtograpsus angulatus* are more dependent on the material in the water column, polychaetes and nematodes on the benthic microalgae, while fishes subsist on a mixture of phytoplankton and benthic microalgae. Phytoplankton, microalgae, and vascular marsh plants therefore all contribute to the organic matter of the estuarine food web, as similarly found in other estua-

rine food web studies (Sullivan & Moncreiff 1990, Currin et al. 1995, Créach et al. 1997, Kwak & Zedler 1997, Riera et al. 1999).

The relative importance of *Spartina* detritus and algal organic matter as a food source is a result of the trade-off between their availability and digestibility. *Spartina* is known to be of lower quality as a food source for organisms given that it has high amounts of indigestible material, but a high productivity provides a large amount of this resource (Mann 1988). In addition, a high proportion of *Spartina* production is transported and accumulated in sediment and can be used as a food source for deposit feeders when enriched by their bacterial biota during decomposition (Mann 1988, Créach et al. 1997). The number of species that uses a source of energy is sometimes considered a measure of its importance for the ecosystem (Sullivan & Moncreiff 1990). However, a source of energy can be particularly important for supporting species with a high biomass and important effects on ecosystem functioning. For example, in this study, *Spartina* supports the dominant species in SW Atlantic estuaries, the burrowing crab *Chasmagnathus granulatus*. These crabs affect sediment dynamics (Botto & Iribarne 2000), the structure of benthic communities (Botto & Iribarne 1999), the feeding activity of shorebirds during low tides (Botto et al. 1998, 2000, Palomo et al. 2003a) and the distribution and feeding activity of juvenile fishes during high tides (Martinetto 2001). Furthermore, at some sites, they have important effects on fiddler crab populations (Daleo et al. 2003) and they can affect the distribution of the fire ant *Solenopsis richteri* in the intertidal zone (Palomo et al. 2003b). Species of *Spartina* are therefore particularly important for SW Atlantic estuarine ecosystems, given that they are the source of energy for *C. granulatus*.

Our results apply to estuaries between southern Brazil and central Argentina. Crustaceans with similar burrowing characteristics and sediment turnover rates that modify intertidal mudflats (e.g. Posey et al. 1991) are common in estuaries worldwide. For example, species of *Callinassa* and *Upogebia* are very common (and dominant) in intertidal mudflats of the western and SE coasts of North America (Suchanek 1985, Posey et al. 1991), in South Africa (Suchanek 1985), in the North Sea (Rowden & Jones 1995) and along the western coast of Scotland (Nickell & Atkinson 1995). Their effect on biogeochemical processes and isotopic signatures should therefore not be neglected.

*Acknowledgements.* We thank D. Harris (UC Davis, Stable Isotope Facility) for isotope analysis. We acknowledge C. H. Peterson, C. Onuf and an anonymous reviewer for their helpful comments on the manuscript. This research was supported by Marine Biological Laboratory (MBL, Woods Hole, Massa-



chusetts) Summer Research Fellowships to F.B. and O.I. This study was partially supported by the Fundacion Antorchas (13956-46 to F.B. and 14116/230 to O.I.). P.M. was supported by a fellowship from CONICET.

## LITERATURE CITED

- Bird EW (1982) Population dynamics of the thalassinidean shrimps and their community effects through sediment modification. PhD thesis, University of Maryland, College Park, MD
- Bortolus A, Iribarne O (1999) Effects of the burrowing crab *Chasmagnathus granulatus* on a *Spartina* saltmarsh. *Mar Ecol Prog Ser* 178:78–88
- Botto F, Iribarne O (1999) Effect of the burrowing crab *Chasmagnathus granulata* on the benthic community of a SW Atlantic coastal lagoon. *J Exp Mar Biol Ecol* 241:263–284
- Botto F, Iribarne O (2000) Contrasting effects of two burrowing crabs (*Chasmagnathus granulata* and *Uca uruguayensis*) on sediment composition and transport in estuarine environments. *Estuar Coast Shelf Sci* 51:141–151
- Botto F, Iribarne O, Martinez M, Delhey K, Carrete M (1998) The effect of migratory shorebirds on the benthic species of three southwestern Atlantic Argentinean estuaries. *Estuaries* 21:700–709
- Botto F, Palomo G, Iribarne O, Martinez MM (2000) The effect of southwestern Atlantic burrowing crabs (*Chasmagnathus granulata*) on habitat use and foraging activity of migratory shorebirds. *Estuaries* 23:208–215
- Cloern JE, Canuel EA, Harris D (2002) Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnol Oceanogr* 47:713–729
- Couch CA (1989) Carbon and nitrogen stable isotopes of meiobenthos and their food resources. *Estuar Coast Shelf Sci* 28:433–441
- Créach V, Schricke MT, Bertru G, Mariotti A (1997) Stable isotopes and gut analyses to determine feeding relationships in saltmarsh macroconsumers. *Estuar Coast Shelf Sci* 44:599–611
- Currin CA, Newell SY, Paerl HW (1995) The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. *Mar Ecol Prog Ser* 121:99–116
- Daleo P, Ribeiro P, Iribarne O (2003) The SW Atlantic burrowing crab *Chasmagnathus granulatus* Dana affects the distribution and survival of the fiddler crab *Uca uruguayensis* Nobili. *J Exp Mar Biol Ecol* 291:255–267
- Deegan LA, Garrit RH (1997) Evidence for spatial variability in estuarine food webs. *Mar Ecol Prog Ser* 147:31–47
- Iribarne O, Bortolus O, Botto F (1997) Between-habitat differences in burrow characteristics and trophic modes in the southwestern Atlantic burrowing crab *Chasmagnathus granulata*. *Mar Ecol Prog Ser* 155:132–145
- Jenkins MC, Kemp WM (1984) Coupling of nitrification and denitrification in two estuarine environments. *Limnol Oceanogr* 29:609–619
- Jennings S, Reñones O, Morales-Nin B, Polunin NVC, Moranta J, Coll J (1997) Spatial variation in the <sup>15</sup>N and <sup>13</sup>C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways *Mar Ecol Prog Ser* 146:109–116
- Kendall C (1998) Tracing nitrogen sources and cycling in catchments. In: Kendall C, McDonnell JJ (eds) *Isotope tracers in catchments hydrology*. Elsevier Science, Amsterdam, p 519–575
- Kwak TJ, Zedler JB (1997) Food web analysis of southern California coastal wetlands using multiple stable isotopes. *Oecologia* 110:262–277
- Lajtha K, Michener R (1994) Introduction. In: Lajtha K, Michener R (eds) *Stable isotopes in ecology and environmental science*. Blackwell Scientific Publishers, Oxford
- Lancaster J, Waldron S (2001) Stable isotopic values of lotic invertebrates: sources of variation, experimental design and statistical interpretation. *Limnol Oceanogr* 46:723–730
- Macko SA, Ostrom NE (1994) Pollution studies using nitrogen isotopes. In: Lajtha K, Michener RM (eds) *Stable isotopes in ecology and environmental science*. Blackwell Scientific Publishers, Oxford, p 45–62
- Mann KH (1988) Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystem. *Limnol Oceanogr* 33:910–930
- Martinetto P (2001) Efecto de los 'cangrejos' de *Chasmagnathus granulata* en el uso de hábitat de cangrejos, peces y zooplancton. In: Iribarne O (ed) *Reserva de biosfera Mar Chiquita características físicas biológicas y ecológicas*. Editorial Martin, Mar del Plata, p 165–170
- McClelland JW, Valiela I (1998) Linking nitrogen in estuarine producers to land-derived sources. *Limnol Oceanogr* 43:577–585
- McClelland JW, Valiela I, Michener RH (1997) Nitrogen-stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. *Limnol Oceanogr* 42:930–937
- Michener RH, Schell DM (1994) Stable isotopes ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener RM (eds) *Stable isotopes in ecology and environmental science*. Blackwell Scientific Publishers, Oxford, p 138–157
- Nickell LA, Atkinson RJA (1995) Functional morphology of burrows and trophic modes of three thalassinidean shrimp species, and a new approach to the classification of thalassinidean burrow morphology. *Mar Ecol Prog Ser* 128:181–197
- Palomo G, Botto F, Navarro D, Escapa M, Iribarne O (2003a) Does the presence of the SW Atlantic burrowing crab *Chasmagnathus granulatus* Dana affect predator-prey interactions between shorebirds and polychaetes? *J Exp Mar Biol Ecol* 290:211–228
- Palomo G, Martinetto P, Perez C, Iribarne O (2003b) Ant predation on intertidal polychaetes of a SW estuarine environment. *Mar Ecol Prog Ser* 253:165–173
- Palomo G, Martinetto P, Iribarne O (2004) Changes in the feeding behavior of the deposit feeder polychaete *Laeonereis acuta* on soft-sediments inhabited by burrowing crabs. *Mar Biol* 145:641–649
- Peterson CH (1977) Competitive organization of the soft-bottom macrobenthic communities of Southern California lagoons. *Mar Biol* 43:343–359
- Peterson CH (1979) Predation, competitive exclusion, and diversity in the soft-sediment benthic communities of estuaries and lagoons. In: Livingston RJ (ed) *Ecological processes in coastal and marine systems*. Plenum Press, New York, p 233–264
- Peterson BJ, Howarth RW, Garrit RH (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227:1362–1363
- Posey MH (1990) Functional approaches to soft-substrate communities: how useful are they? *Aquat Sci* 39:343–356
- Posey MH, Dumbauld BR, Armstrong DA (1991) Effects of a burrowing mud shrimp, *Upogebia pugettensis* (Dana), on abundance of macro-infauna. *J Exp Mar Biol Ecol* 148:283–294

- Rhoads DC, Young DK (1970) The influence of deposit feeding organisms on sediment stability and community trophic structure. *J Mar Res* 28:150–178
- Rice DL, Rhoads DC (1989) Early diagenesis of organic matter and the nutritional value of sediment. In: Lopez G, Taghon G, Levinton J (eds) *Lecture notes on coastal and estuarine studies*, Vol 31. Springer-Verlag, New York, p 59–97
- Riera P, Stal LJ, Nieuwenhuize J, Richard P, Blanchard G, Gentil F (1999) Determination of food sources for benthic invertebrates in a salt marsh (Aguillon Bay, France) by carbon and nitrogen stable isotopes: importance of locally produced sources. *Mar Ecol Prog Ser* 187:301–307
- Rowden AA, Jones MB (1995) The burrow structure of the mud shrimp *Callinassa subterranea* from the central North Sea. *J Nat Hist* 29:1115–1165
- Rowden AA, Jago CF, Jones MB (1998) Influence of benthic macrofauna on the geotechnical and geophysical properties of surficial sediment, North Sea. *Cont Shelf Res* 18:1347–1363
- Suchanek TH (1985) Thalassinidean shrimps burrows: ecological significance of species-specific architecture. *Proc Int Coral Reef Cong Tahiti* 5:205–210
- Sullivan MJ, Moncreiff CA (1990) Edaphic algae are an important component of salt marsh food webs: evidence from multi stable isotope analysis. *Mar Ecol Prog Ser* 121:99–116
- Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: control and ecophysiological and ecosystem consequences. *Limnol Oceanogr* 42:1105–1118
- Zar JH (1999) *Biostatistical analysis*, 3rd edn. Prentice Hall, Englewood Cliffs, NJ
- Ziebis W, Forster S, Huettel M, Jorgensen BB (1996) Complex burrows of the mud shrimp *Callinassa truncata* and their geochemical impact in the sea bed. *Nature* 382:619–622

*Editorial responsibility: Charles Peterson (Contributing Editor), Morehead City, North Carolina, USA*

*Submitted: July 23, 2004; Accepted: December 22, 2004  
Proofs received from author(s): May 4, 2005*