

Positive interactions of the smooth cordgrass *Spartina alterniflora* on the mud snail *Heleobia australis*, in South Western Atlantic salt marshes

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Abstract

The role of positive interactions is often crucial in communities with intense abiotic stress such as intertidal environments. Grasses acting as ecosystem engineers, for example, may ameliorate intertidal harsh physical conditions and modify the community structure. The mud snails *Heleobia australis* d'Orbigny frequently inhabit the SW Atlantic marshes, mainly associated to intertidal marsh plants (mainly the smooth cordgrass *Spartina alterniflora* Loisel) probably due to the plant indirect effects. The purpose of this work was to investigate the magnitude of these association and the processes that generate the pattern. Samples of the snail abundance in six SW Atlantic coastal marshes show that *H. australis* is associated to coastal areas of low energy and low or none freshwater input. This result is important because this species is being used as bioindicator of coastal estuarine systems during the Holocene. Thus the paleontological interpretation based on this species should be revised. Within the studied areas, snails are associated to intertidal marsh plants. However, stable isotope analysis shows that neither plant nor their epiphytes are their main food sources. Field experiments show that snails actively select areas with plants, although tethering experiments show that plants do not provide shelter from predators. However, plants do buffer physical stress factors such as temperature, which generate important mortality outside plants covered areas. These positive interactions have large effects on *H. australis* distributions in marsh communities; increasing the habitats available for colonization and affecting their local distribution.

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1. Introduction

Organisms that by their own presence modify the physical conditions of their environment are named autogenic ecosystem engineers (Jones et al., 1994, 1997). Their environmental changes can have strong effects on the community structure (Jones et al., 1997; Stachowicz, 2001) and, in many cases with positive

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effects on the species abundance and richness (i.e., Stachowicz, 2001; Wright et al., 2004). Positive or facilitative interactions may be direct such as the reduction of thermal stress via shading, or indirect such as the removal of competitors or predators (Stachowicz, 2001; Bruno et al., 2003). The role of positive interactions is often crucial in communities with intense abiotic stress such as deserts, alpinas and salt marshes and their importance often increase with stress intensity (e.g., Bertness and Hacker, 1994; Stachowicz, 2001; Callaway et al., 2002; Bruno et al., 2003).

Given that salt marshes are harsh physical environments, positive interactions are very important forces affecting their community structure (e.g., Bertness and Hacker, 1994; Stachowicz, 2001). Saltmarshes are highly productive, often being the basis of estuarine food webs (Day et al., 1989), however they are also stressful environments. This is because they are exposed to the tides, which increase sediment salt accumulation and generate drastic changes in temperature (Adam, 1993). However, marsh plants may ameliorate harsh physical microenvironmental conditions, favoring the establishment of other organisms by shading (i.e., Bertness and Hacker, 1994;

Callaway, 1995; Bortolus et al., 2002) or providing predation refuge (i.e., Hovel et al., 2001; Lewis and Eby, 2002) affecting directly and indirectly marsh community structure.

Hydrobioid snails are organisms frequently found inhabiting coastal environments, (e.g., Lillebo et al., 1999; De Francesco and Isla, 2004) and may attain high densities and biomass, often constituting the main group of benthic macroinvertebrates in aquatic ecosystems (Lillebo et al., 1999). Their distributions are affected by biological factors such as interspecific competition (Cherrill and James, 1987; Grudemo and Bohlin, 2000), parasitism (Mouritsen and Jensen, 1994), and predation (Barnes and De Villiers, 2000), or environmental factors such as salinity (Hylleberg, 1986; De Francesco and Isla, 2003), temperature (Lassen, 1979; Lassen and Clark, 1979), wave exposure (Fenchel, 1975a; Cherril and James, 1985), type of sediment (Fenchel, 1975b; Grudemo and Johannesson, 1999), tolerance to anoxia and desiccation (Fenchel, 1975a). Thus, they are prone to have their spatial distribution shaped by the activity and distribution of other species (mainly ecosystem engineering).

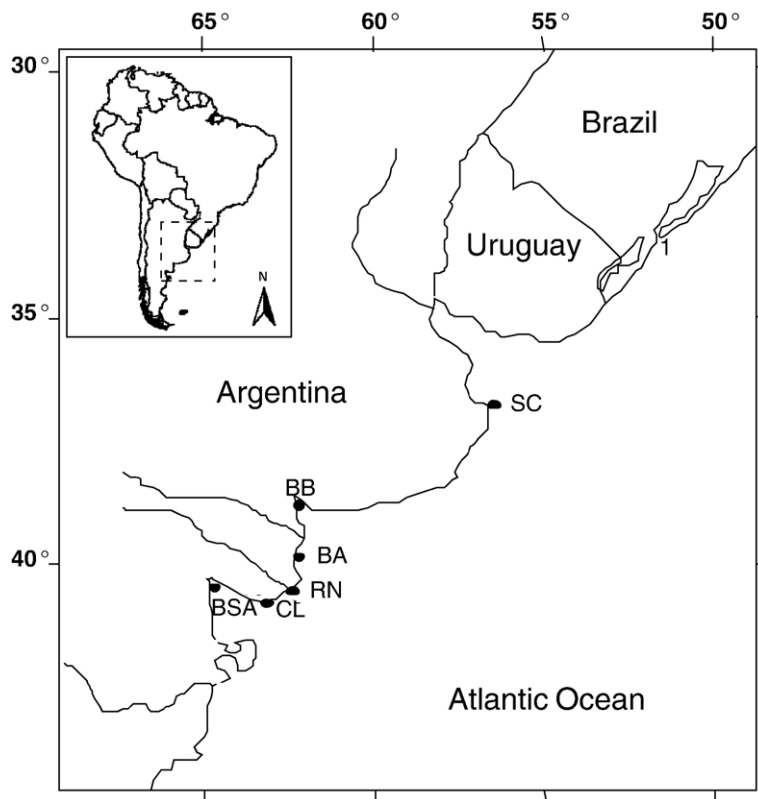


Fig. 1. Marshes sampled through the south-west Atlantic coast. SC: San Clemente ($36^{\circ} 22' S$, $56^{\circ} 45' W$), BA: Bahía Anegada ($39^{\circ} 48' S$, $40^{\circ} 42' W$), BB: Bahía Blanca ($38^{\circ} 41' S$, $39^{\circ} 30' W$), RN: Río Negro ($41^{\circ} 00'$), CL: Caleta de los Loros ($41^{\circ} 01'$), BSA: Bahía San Antonio ($40^{\circ} 42' S$, $40^{\circ} 50' W$).

Indeed, in the SW Atlantic, the hydrobioid mud snail *Heleobia australis* d'Orbigny is frequently found in salt marshes (pers. obs.), where cordgrasses (*Spartina* spp.) are the dominant plant (Bortolus and Iribarne, 1999; Isacch et al., 2006). This snail has a broad geographic distribution from Rio de Janeiro (Brazil, 22° 54' 10" S) to San Antonio Oeste (northern Argentinean Patagonia, 40° 84' 59" S; Aguirre and Farinati, 2000), distributed coincident with the limits of tidal influence (Darrigran, 1995; De Francesco and Isla, 2003; Elias et al., 2004). Moreover, shells of *H. australis* are present in high abundance and good preservation in Holocene successions cropping out along the northeastern coast of Argentina (De Francesco and Zarate, 1999). They were described as typically associated to mixohaline conditions (Aguirre and Urrutia, 2002), and used as a bioindicator to reconstruct coastal environments and sea level changes during the Holocene (De Francesco and Zarate, 1999; Aguirre and Farinati, 2000; Aguirre and Urrutia, 2002). Although their zonation patterns has been preliminary studied (Darrigran, 1995; De Francesco and Isla, 2003), this association has been inferred only from fossil deposits (Aguirre and Urrutia, 2002; Aguirre et al., 2002) and from estuarine habitat studies (e.g., coastal lagoon, or stream and their estuarine area of influence, Bemvenuti et al., 1978; Capitoli et al., 1978; Albertoni et al., 2001; De Francesco and Isla, 2003, 2004). However, the biological variables influencing alive *H. australis* distribution remain unknown (De Francesco and Isla, 2001, 2004) and comparative samples with marine habitats were never performed.

Moreover, our previous observations suggest that *H. australis* are mainly associated to marsh plants in low-energy coastal environments (mainly hypersaline). Thus, the purpose of this work is 1) to identify large patterns of distribution of the intertidal mud snail *H. australis* in the SW Atlantic and correlate this distribution with environmental characteristics through comparative samplings, then 2) through sampling and field experiments we evaluated their distribution in relation to intertidal marshes of the cordgrass *Spartina alterniflora* Loisel, and some of the hypothesis that may explain their distribution pattern.

2. Materials and methods

2.1. Study area

The study was performed in coastal marshes from the mouth of La Plata River (Ria de San Clemente; 36° 22' S), to the northern Patagonia (San Antonio bay, 40° 84' 59" S) including most of the Argentinean marshes where *S. alterniflora* overlaps with the distribution range of *H. aus-*

tralis (see Fig. 1), which also include most south-western Atlantic coastal marshes (see Isacch et al., 2006). Marsh sediments show variable quantities of sand, shells, and boulders, but mud always present the highest percentage (Calliari, 1998; Yorio, 1998). Two groups of marshes can be identified in this region (see Isacch et al., 2006): those associated with brackish waters (dominated by *S. densiflora* Brongniarti), and those related to marine waters (dominated by *S. alterniflora*). Cordgrasses *S. alterniflora*, *S. densiflora* and the perennial glasswort *Sarcocornia perennis* (P. Miller) A.J. Scott. (formerly known as *Salicornia ambigua* Michx) cover 70% of saltmarshes of the south-western Atlantic coast (Isacch et al., 2006). Given logistic and economic constrains, experiments and other samplings to understand the relationship between snails and plants were performed at the low marsh of Bahía Blanca (38° 41' S, see Fig. 1), located on a mesotidal system affected by up to 4 m tides (Perillo and Piccolo, 1991). Experiments and samplings were performed during the cold season (winter or autumn) and repeated during the warm seasons (spring or summer), given that temperature range in this region goes from -3.1 °C in cold season up to 37.9 °C in the summer (Piccolo and Diez, 2004), with potential for affecting distribution of intertidal species.

2.2. Large and small scale patterns of distribution of *H. australis*

To identify large scale patterns of *H. australis* distribution, we sampled their distribution in most of the Argentinean coastal low-energy areas with marshes (see Fig. 1). This sampling scheme includes the two marsh groups in this region, brackish (San Clemente, thereafter SC, and Rio Negro, thereafter RN) and marine marshes (Bahia Anhegada, thereafter BA; Bahia Blanca, thereafter BB; Caleta de Los Loros, thereafter CL, and Bahia San Antonio, thereafter, BSA; see Isacch et al., 2006 and Fig. 1), which then allowed us to associate snail distribution with environmental characteristics (i.e., freshwater input, sediment characteristics). Also, we explored if *H. australis* abundance is affected by the distribution of *S. alterniflora* marshes. With this purpose, we sampled presence and density of *H. australis* in each site during the low tide, by collecting core samples (10 cm diameter and 2 cm deep) inside and outside the marsh (10 replicates in each one) at the same tidal height and within the same general region (separated by no more than 20 m). Samples were sieved through a 0.5 mm mesh screen and *H. australis* were counted under 10× magnification. The null hypothesis of no difference in *H. australis* densities between habitats was evaluated by a *t*-test (Zar, 1999) for each location.

To explore the hypothesis that *H. australis* distribution is related to *S. alterniflora* stems, we recorded *H. australis* density on three microhabitats located (1) next to *S. alterniflora* stems (less than 5 cm), (2) between *S. alterniflora* stems (between 5 and 10 cm, approximately the maximum distance observed among stems is 20 cm approximately), and (3) in unvegetated areas (areas without grass cover, and separated at least 50 cm to vegetated areas). Densities were estimated in May 2006 and January 2007 by collecting in each microhabitat 10 core samples (5 cm diameter and 2 cm deep). Samples were sieved through a 0.5 mm mesh screen and snails were counted under 10× magnification. The null hypothesis of no differences in *H. australis* densities in the three microhabitats was evaluated by an ANOVA for each sampling date. A posteriori Tukey tests were used to identify differences among treatments with an acceptance level for significance of 0.05 (Zar, 1999).

2.3. Processes that affect small scale distribution pattern of *H. australis*

To experimentally evaluate if *S. alterniflora* affects *H. australis* presence in the marsh, in May 2006 and in January 2007 we established 20 plots (0.6 m side), assigned to two different treatments: (1) Removal, plots in which all *S. alterniflora* stems were cut to the ground, resulting in areas without *S. alterniflora* cover ($n=10$); (2) Control, plots in which we left the natural *S. alterniflora* cover ($n=10$). After 30 days, we collected a core sample from the center of each plot. The null hypothesis of no differences in *H. australis* densities with and without plant cover was evaluated by means of a *t*-test (Zar, 1999) for each sampling date.

S. alterniflora stems generate microhabitats that favor the establishment of several species (Callaway, 1995; Bertness and Hacker, 1994), thus they are actively selected by a variety of species (Lewis and Eby, 2002). Thus to test the hypothesis that *H. australis* actively select microhabitats next to *S. alterniflora* stems, in March 2006 and May 2006 we conducted experiments manipulating snail numbers. To diminish the incidental movement of snails related to water circulation, we placed 10 circular cages of 10 cm diameter (to avoid overlap among microhabitats) and 3 cm above the sediment (which allow snail movement outside the box) at the three different marsh habitats. All *H. australis* inside the cages were initially removed and then, 10 live individuals randomly chosen were introduced in each cage; after 24 h we recorded the number of *H. australis* in each cage. ANOVA followed by Tukey tests (Zar, 1999) were used

to evaluate the null hypothesis of no differences in *H. australis* numbers between microhabitats.

S. alterniflora stems reduce predation rates of several intertidal species (Hovel et al., 2001; Lewis and Eby, 2002), which also may be the case with *H. australis*. To evaluate this hypothesis, the proportion of *H. australis* preyed was evaluated using tethering experiments. Although tethering cannot be used to measure absolute predation rates, it is useful to compare relative predation rates at different sites, given that experimental artifacts can be considered constant across sites (e.g., Peterson and Black, 1994; Aronson et al., 2001; Haywood et al., 2003). Live *H. australis* (randomly captured from an area immediately adjacent to the experimental area) were tethered in each of the three marsh microhabitats (10 in April 2006 and 20 in February 2007 for each treatment). One end of a 5 cm monofilament nylon (0.05 mm diameter) tether was attached to *H. australis* shells using cyanoacrylate adhesive, and the other end of the tethers was attached to grounded metal stakes, which were separated from each other by at least 50 cm. The length of monofilament nylon (5 cm) was selected in order to avoid snail entangling to *Spartina* stems (max distance between stems is 20 cm approximately) and thus snails could move in similar way in each of the three marsh microhabitats. After 48 h, we collected all tethered *H. australis* and recorded the proportion of absences of these snails as an estimator of predation rates (i.e., Warren, 1985; Hovel et al., 2001; Kempainen et al., 2005). Snails lost by predation were scored when an individual was missing and a piece of shell remained glued to the nylon (e.g., Fernandez et al., 1993). When no pieces of the shell remained glued we considered data as lost and we did not use it to estimate predation rates, given that the predation event was not clear (i.e., snails may have escaped). Chi-Square test (Zar, 1999) was used to evaluate the null hypothesis of no differences in snails lost or preyed among microhabitats for each sampling date.

Habitats near marsh grasses may be selected because the grass or their epiphyte may provide food sources for *H. australis*. To test the hypothesis that *S. alterniflora* indirectly provide food resources (as epiphytes) to the *H. australis*, in May 2006 and January 2007 we conducted experiments manipulating the epiphytes. Epiphytes in the study area are distributed approximately up to 15 cm high on *S. alterniflora* stems (pers. obs). Twenty randomly selected stems were assigned to two different treatments: (1) epiphyte removal: we brushed and cleaned 10 *S. alterniflora* stems with marsh water to remove epiphytes, and (2) control: 10 stems with natural epiphytes. After 48 h, we collected core samples next to the base of each

stem. The null hypothesis of no differences in *H. australis* densities between treatments was evaluated with *t*-tests (Zar, 1999). Additionally, we performed stable isotope analysis (Peterson et al., 1985; Michener and Schell, 1994) to evaluate if *H. australis* feed on sediment, on *S. alterniflora*, or on their associated epiphytes. Five samples of *H. australis*, *S. alterniflora*, epiphytes and sediments were taken. Snail samples were pools of 20 individuals without shells. Plant samples (a pool of 5 leaves and stems of 5 different plants) were extracted by hand. We extracted epiphytes from stems by cleaning each stem with distilled water and collecting the material in previously combusted filters (500 °C during 2 h). Surface sediment was extracted with a core 10 cm diameter and 2 cm deep. Snail, sediment, and plants were dried and milled to a fine powder. Carbonate was extracted from sediment by fuming HCl during 24 h. All C and N samples were analyzed at the Stable Isotope Facility of the University of California (Davis). Then, we performed a mixing model (Phillips and Gregg, 2001) to calculate the mean proportion (*f*) of each of the three sources: sediment (sed), epiphytes (ep), and *S. alterniflora* (Sp), which contribute to the *H. australis* diet using the following mass balance equations:

$$\delta^{13}\text{C } H. \text{ australis} = f_{\text{sed}}\delta^{13}\text{C } \text{sed} + f_{\text{ep}}\delta^{13}\text{C } \text{ep} + f_{\text{Sp}}\delta^{13}\text{C } \text{Sp}$$

$$\delta^{15}\text{N } H. \text{ australis} = f_{\text{sed}}\delta^{15}\text{N } \text{sed} + f_{\text{ep}}\delta^{15}\text{N } \text{ep} + f_{\text{Sp}}\delta^{15}\text{N } \text{Sp}$$

$$f_{\text{sed}} + f_{\text{ep}} + f_{\text{Sp}} = 1$$

For our purposes, the isotopic signature for the mixture was adjusted to account for fractionation assuming a fractionation of 3.4 (Michener and Schell, 1994). We also approximate SEs, for estimates of source proportions following Phillips and Gregg (2001). These formulas take into account the isotopic signature variability of both the sources and the mixture (Phillips and Gregg, 2001).

Marsh plants may ameliorate harsh physical microenvironmental conditions by decreasing thermal stress (i.e., Bertness and Hacker, 1994; Callaway, 1995; Bortolus et al., 2002). The range of air temperature in this region goes from −3.1 °C in cold season up to 37.9 °C in the summer seasons (data from 1999–2001 period, Piccolo and Diez, 2004), thus with potential for affecting distribution of intertidal species. *S. alterniflora* shade may ameliorate harsh physical microenvironmental conditions for intertidal species, which can also be affecting the distribution of *H. australis*. To test the hypotheses that *S. alterniflora* directly ameliorate thermal stress providing shade for *H. australis*, in May 2006 and January 2007 we measured temperatures in the three microhabitats. We collected temperature data on 10 randomly selected soil samples (2 cm deep) in the 3 different microhabitats of the

S. alterniflora marsh. Tukey tests after ANOVA (Zar, 1999) were used to evaluate the null hypothesis of no differences in soil temperature among microhabitats of the *S. alterniflora* marsh. Also, to evaluate if *H. australis* were buried in the sediment to evade higher soil temperature on the surface, five cores (10 cm diameter and 10 cm deep) were obtained at low tides when the sunlight was strongest (between 12:00 and 15:00 h) inside and outside the marsh. The samples were divided in situ in 3 layers of depth (2 cm, 5 cm and 10 cm), and processed then as previously described. Tukey tests after ANOVA (Zar, 1999) were used to evaluate the null hypothesis of no difference in abundance of *H. australis* between sediment depths for each habitat. Additionally, in February 2006 and May 2006 experiments manipulating shade intensity were conducted. Twenty plastic laminates (10 × 20 cm) were placed vertical, and assigned to two different treatments: (1) Shade: 10 plastic laminate were placed in a North–South orientation (perpendicular to sunlight when it was stronger) resulting in more shade than (2) Light: 10 plastic laminates were placed in an E–W orientation (parallel to sunlight when it was stronger). After 48 h, we collected a soil core in the center base of each laminate. The null hypothesis of no differences in *H. australis* densities between treatments was evaluated with a *t*-test (Zar, 1999) for each sampling date. Also to test the hypothesis that shadows of *S. alterniflora* stems directly affect the survival rate of *H. australis*, the proportion of dead *H. australis* was evaluated using a tethering experiment as described before. After 48 h, we collected

Table 1
Heleobia australis densities (mean and SD) in the southwest Atlantic coast

Sites	groups	Soil	<i>Heleobia australis</i> densities (snails cm ⁻²)		<i>t</i> -test		
			Tidal plain	Marsh	<i>t</i>	df	<i>p</i> -value
SC	Brackish	Mud	Mean±SD	Mean±SD			
BB	Marine	Mud	0.0	0.0			
BA	Marine	Mud and sand	0.7±0.4	1.8±0.5	−4.0	18	<0.05
RN	Brackish	Mud	0.0	0.0			
CL	Marine	Mud and sand	1.1±1.7	5.7±1.5	−5.2	18	<0.05
BSA	Marine	Boulders	0.0	0.01±0.01	−2.5	18	<0.05

SC: San Clemente, BB: Bahía Blanca, BA: Bahía Anegada, RN: Río Negro, CL: Caleta de los Loros, BSA: Bahía San Antonio, (see Fig. 1). *t*-values from comparison of marsh and tides snail densities at each site (*p*<0.05).

all snails remaining tethered and recorded the proportion of dead snails as an estimator of mortality rate induced by abiotic stress. Chi-Square test (Zar, 1999) was used to evaluate the null hypothesis of no differences in the mortality rates among microhabitats of the *S. alterniflora* marsh.

3. Results

3.1. Large and small scale patterns of distribution of *H. australis*

H. australis was only present in intertidal environments with more influence of marine waters (Table 1; Fig. 1). Also, densities were much higher in the marsh habitats than in the bare tidal flats (Table 1) in all sites (see Fig. 1).

H. australis densities were always different among *S. alterniflora* microhabitats (May 2006: ANOVA, $F_{2,27}=36.70$, $p<0.05$, Fig. 2A; January 2007: ANOVA, $F_{2,27}=24.35$, $p<0.05$, Fig. 2B) with higher densities next

to stems than between stems, and no *H. australis* were found on unvegetated areas (Fig. 2B).

3.2. Processes that affect small scale distribution pattern of *H. australis*

Both times in which we removed *S. alterniflora* cover, *H. australis* densities diminished up to 97% (May 2006: $x=0.05$, S.D.=0.10 snails cm^{-2} ; January 2007: $x=0.01$, S.D.=0.03 snails cm^{-2}) compared to controls (May 2006: $x=1.90$, S.D.=0.70 snails cm^{-2} , $t_{18}=8.30$, $p<0.05$; January 2007: $x=0.39$, S.D.=0.15 snails cm^{-2} , $t_{18}=7.75$, $p<0.05$).

Also, 24 h after we homogenized *H. australis* densities in experimental cages in the three microhabitats, *H. australis* densities varied among treatments (January 2006: ANOVA, $F_{2,27}=4.58$, $p<0.05$; April 2006: ANOVA, $F_{2,27}=60.20$, $p<0.05$). Densities of *H. australis* in treatments next to ($x=0.09$, S.D.=0.04 snails cm^{-2}) and between stems ($x=0.09$, S.D.=0.03 snails cm^{-2}), were higher than in areas without stems ($x=0.04$, S.D.=0.02

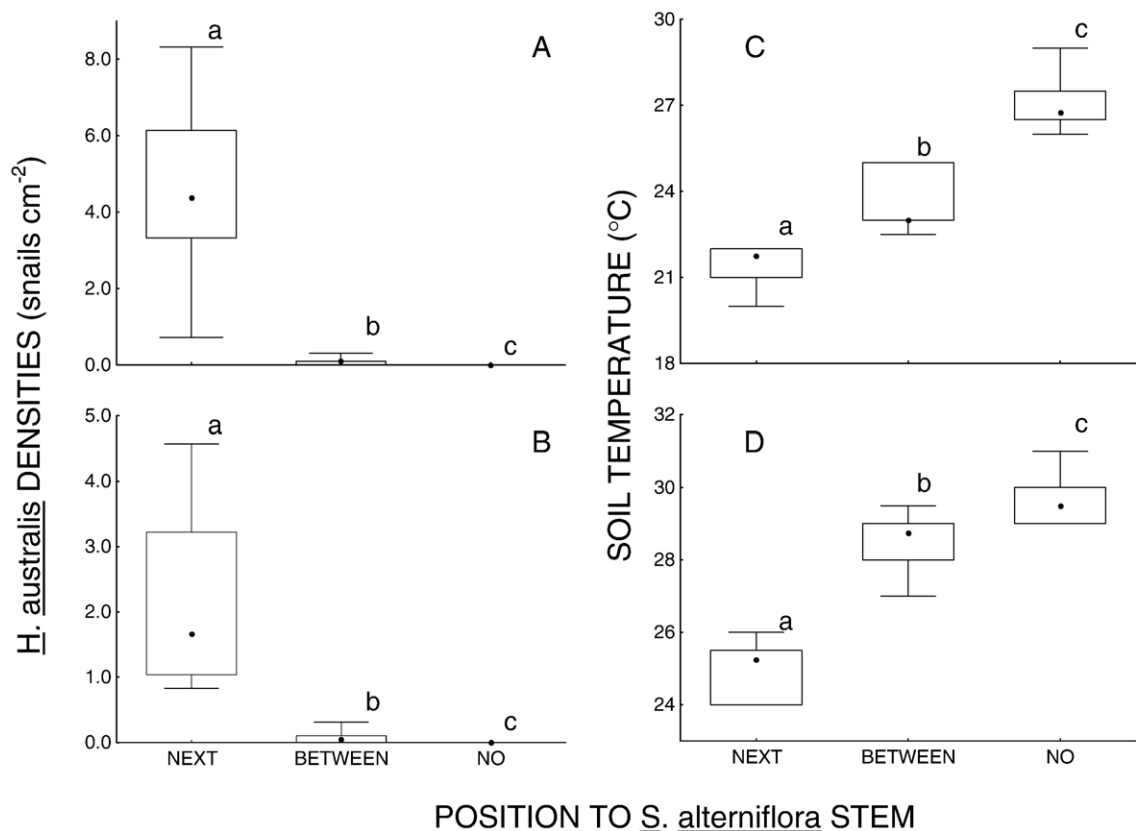


Fig. 2. Natural densities of *H. australis* at each microhabitat of *S. alterniflora* (Right) and soil temperature of the three microhabitat of *S. alterniflora* marsh (Left) in May 2006 (A and C), and January 2007 (B and D). “Next”: next to *S. alterniflora* stems, “between”: between stems and “no”: areas without stems. Limits of the box represent 25 and 75 percentiles, vertical lines represent minimums and maximums and black points inside the boxes represent the median values. Different letters indicated significant differences ($p<0.05$) Tukey test after ANOVA.

snails cm^{-2} , January: Tukey test, $p < 0.05$ for both comparisons). Similar results were registered in April 2006 (ANOVA, $F_{2, 27} = 60.20$, $p < 0.05$). Densities of *H. australis* in treatments next to ($x = 0.10$, S.D. = 0.02 snails cm^{-2}) and between stems ($x = 0.12$, S.D. = 0.01 snails cm^{-2}), were higher than in areas without stems ($x = 0.02$, S.D. = 0.02 snails cm^{-2} , Tukey test, $p < 0.05$ for both comparisons).

The number of *H. australis* remaining per microhabitat type in the tethering experiment in April 2006, was not different after 48 h (Chi-Square = 0.39, $df = 1$, $p > 0.50$ for both comparisons). Only one *H. australis* tethered next to stems ($n = 10$) and another one tethered far from stems ($n = 10$) were lacking after 48 h. Similar results were registered in February 2007 (Chi-Square = 0.36, $df = 1$, $p > 0.60$ for both comparisons), with only 1 *H. australis* tethered next to stems ($n = 20$), two *H. australis* tethered far from stems ($n = 20$), and two tethered between stems ($n = 20$) lacking after 48 h. In the two tethering experiments, all absent snails showed no piece of the glued shell remaining attached to the nylon, suggesting that they were missing rather than eaten (e.g., Fernandez et al., 1993).

There were no differences in *H. australis* densities next to *S. alterniflora* stems with (March 2006: $x = 0.15$, S.D. = 0.12 snails cm^{-2} ; January 2007: $x = 0.57$, S.D. = 0.47 snails cm^{-2}) and without epiphytes (March 2006: $x = 0.31$, S.D. = 0.34 snails cm^{-2} , $t_{18} = -0.79$, $p = 0.44$; January 2007: $x = 0.84$, S.D. = 1.13 snails cm^{-2} , $t_{18} = -0.69$, $p = 0.49$). The isotopic analysis shows that the sediment is the main food source of *H. australis* with a mean contribution of 75%. The mean contribution of epiphytes is around 28% and *S. alterniflora* compromises only 4% of snails diet (Fig. 3).

The soil temperature was different among microhabitats (ANOVA, $F_{2,18} = 100.00$, $p < 0.01$). Soil temperature next to stems was lower, than between or than without stems (Fig. 2C). Similar results were registered in January 2007 (ANOVA, $F_{2,18} = 94.16$, $p < 0.01$), with soil temperature next to stems lower than between or than without stems (Fig. 2D). Cores sampled during the low tide and when the sunlight was stronger, showed that *H. australis* was buried up to 2 cm deep in the sediment. Densities of *H. australis* at 2 cm deep ($x = 2.20$, S.D. = 0.5 snails cm^{-2}) were higher than at 5 cm deep ($x = 0.02$, S.D. = 0.02 snails cm^{-2} , Tukey test, $p < 0.05$) and *H. australis* were not found at 10 cm deep (Tukey test, $p < 0.05$).

When we modified the shade area, *H. australis* densities were higher in soil areas protected of sunlight (February 2006: $x = 0.80$, S.D. = 0.70 snails cm^{-2} ; March 2006: $x = 1.20$, S.D. = 1.00 snails cm^{-2}) than in exposed areas (February 2006: $x = 0.16$, S.D. = 0.17 snails cm^{-2} , $t_{18} = 3.2$, $p < 0.05$; March 2006: $x = 0.40$, S.D. = 0.2 snails cm^{-2} , $t_{18} = 2.18$, $p < 0.05$). The tethering experiments showed that the percentage of dead *H. australis* in each habitat was higher in areas without *S. alterniflora* (67%) than in areas between stems (33%, Chi-Square = 4.00, $df = 1$, $p < 0.05$), and than in areas next to stems (5%, Chi-Square = 4.70, $df = 1$, $p < 0.05$).

4. Discussion

Our results show that *H. australis* is associated to marine habitats, and within these environments it is mainly associated to *S. alterniflora* dominated marshes. *H. australis* actively selected vegetated areas of marshes, and resulted in a joint distribution between both species.

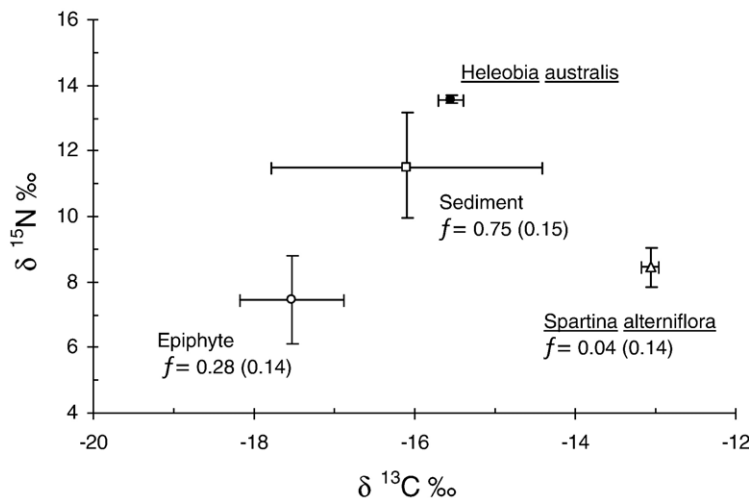


Fig. 3. Mean (\pm SD) isotope values for the *H. australis* and the three possible food sources. For each source we also reported the mean and standard errors (SE) of the proportions (f) calculated following Phillips and Gregg (2001).

Although predation hypothesis cannot be rejected, our results suggest that predation is a weak force to explain the association between *H. australis* and *S. alterniflora*. Also, this association was not explained by epiphytes availability on *S. alterniflora* stems (food supply hypothesis). Moreover, the stable isotopic analysis shows that *S. partina alterniflora* does not directly contribute to the diet of *H. australis*, being the sediment the main food source with lower importance of epiphytes. Our study suggests that temperature and dehydration stress may regulate *H. australis* distribution and survival through the marshes due to a reduction in soil temperature by the shadow provided by *S. alterniflora* stems.

Our comparative samples show that *H. australis* was associated to marine influenced salt marshes (dominated by *S. alterniflora*) rather marshes with high fresh water inputs. These results have implications for paleoenvironmental reconstruction, given that *H. australis* distribution has been widely associated and restricted to estuaries influenced by tidal excursion environments (Darrigran, 1995; De Francesco and Zarate, 1999; Aguirre and Farinati, 2000; Aguirre and Urrutia, 2002). Based on such results, *H. australis* have been used as indicators of paleosalinity to reconstruct coastal environments and sea level changes during the Holocene, with its optimum in brackish (mixohaline) conditions (i.e., estuarine conditions, not typically marine habitats, De Francesco and Zarate, 1999; Aguirre and Farinati, 2000; Aguirre and Urrutia, 2002). However, this information was based on snail abundances in salinity gradient of estuarine habitats (Albertoni et al., 2001; De Francesco and Isla, 2003, 2004), from fossil snail association with estuarine habitats (Aguirre and Urrutia, 2002; Aguirre et al., 2002), or from bibliographic compilation of the same former works (Aguirre and Urrutia, 2002; Aguirre et al., 2002). However, our evidences are different. Although we cannot reject its presence in other brackish marshes, among the examined sites this species is mostly associated to mud, high salinity, and low energy coastal areas, rather than to boulders and brackish areas. Moreover, within these habitats they are strongly associated to *S. alterniflora* marshes.

Given that *H. australis* actively selected marsh vegetated areas, and within vegetated areas, actively preferred the areas next to *S. alterniflora* stems, this resulted in a joint distribution between both species. Marsh plants are likely to increase habitat availability for many organisms by directly diminishing predation rates (i.e., snail predation by fish, crabs, and birds, Vaughn and Fisher, 1988; Hovel et al., 2001; Lewis and Eby, 2002). For example, the periwinkle *Littoraria irrorata* of intertidal marshes from USA, remains above

the water surface during high tides, and close to the bases of *S. alterniflora* stems at low tides, reducing its vulnerability to predators such as crabs *Callinectes sapidus*, killifishes *Fundulus* spp. and birds (Warren, 1985; Vaughn and Fisher, 1988; Hovel et al., 2001; Lewis and Eby, 2002). In our study area, potential predators for *H. australis* are fish such as mouth croaker *Micropogonias furnieri*, which are active during high tides in the marsh areas (Martinetto et al., 2005) or shorebirds (Botto et al., 1998), which are active during low tides. Although predation cannot be rejected, and it is possible that some predators occasionally may feed on *H. australis*, our results suggest that predation is a weak force affecting *H. australis* distribution in these marshes.

The association of *H. australis* and *S. alterniflora* stems is not explained by food supply, given that the presence/absence of epiphytes in stems, did not affect *H. australis* densities next to stems. Also, the stable isotopic ratios of *H. australis* suggested that this snail is not using *S. alterniflora* or epiphytes as a main food source but forage mainly on sediments. This species is probably taking the benthic microalgae (i.e., Albertoni et al., 2001; Figueiredo-Barros et al., 2006) growing on marsh soil. Therefore, there is no evidence that the restricted pattern of *H. australis* distribution is due to the food supply.

Marsh plants are likely to increase habitat availability for many organisms by directly helping them to overcome stressful temperature conditions (e.g., Bortolus et al., 2002 for crabs; Hovel et al., 2001 for snails). Shade provided by *S. alterniflora* stems heavily reduced soil temperatures. Also, given that a higher proportion of dead *H. australis* were experimentally found in areas without stems in February 2007 (maximum daily temperature reached 35 °C), it suggests that temperature and dehydration stress may regulate *H. australis* distribution and survival through the marshes. Temperature and dehydration stress often regulate intertidal organisms distribution and survival (e.g., Nomann and Pennings, 1998; Bertness, 1999; Bortolus et al., 2002), and positive interactions usually diminish physical stress, favoring the survival of organisms living there (e.g., Callaway, 1995; Bortolus et al., 2002). It has been reported that marsh grass ameliorates harsh and limited physical microenvironmental conditions, favoring the establishment of other organisms (e.g., Bertness and Hacker, 1994; Bruno, 2000a,b for perennial plants and salt grass; Nomann and Pennings, 1998; Bortolus et al., 2002, for crabs; Hovel et al., 2001 for snails). Vegetated and clear areas may differ in reflectivity, heat capacity and conductance, and the amount of heat lost through evaporation (Bazzaz, 1998). Plant cover tends to

condense water as well as to diminish evaporation below it by shading the understory (Callaway, 1995). Thus, *H. australis* seems to avoid bare areas and aggregate next to *S. alterniflora* stems to avoid high soil temperatures (thermal stress hypothesis). Other snails, as the mangrove whelk snail, *Telescopium telescopium*, also aggregate under mangrove trees during long periods of aerial exposure to avoid both desiccation and heat stress (Lasiak and Dye, 1986). *Littoraria irrorata* also climb to stems to thermoregulate and avoid stress and desiccation (Vaughn and Fisher, 1992). Despite this, no agreement exists about which factor (thermal stress or predation rates) was the strongest one and which was the additional benefit of this behavior (Vaughn and Fisher, 1992). Recent results suggest that predator-induced mortality may be a much stronger selective factor for *L. irrorata* selecting stems of marsh grass, than physiological stress (Henry et al., 1993; Hovel et al., 2001). In our study, the fact that *H. australis* established more in the soil shade by plastic laminates, than in soil laminates exposed to sun light, and the high mortality registered during the second tether experiment (when sun irradiance was more intense), suggested that thermal stress rather than predation rates or food supply hypotheses was the key factor in the selection of stems microhabitat for *H. australis* on marsh.

In summary, our results show that *S. alterniflora* positively drive the spatial distribution of *H. australis*. Although predator avoidance by *H. australis* cannot be rejected as an additional benefit, the facilitation mechanism seems to be through the stems of *S. alterniflora* buffering *H. australis* from physical stress factors (i.e., temperature). These positive interactions can have large effects on *H. australis* distributions within marsh communities; increasing the habitats available for colonization and affecting their local distribution.

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