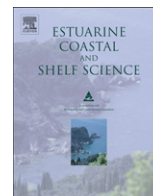




Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

The Southwest Atlantic intertidal burrowing crab *Neohelice granulata* modifies nutrient loads of phreatic waters entering coastal area

Eugenia Fanjul ^{a,b,*}, María A. Grela ^{b,c}, Alejandro Canepuccia ^{a,b}, Oscar Iribarne ^{a,b}

^aLaboratorio de Ecología, Departamento de Biología (FCEyN), Universidad Nacional de Mar del Plata, CC 573 Correo Central, B7600WAG Mar del Plata, Argentina

^bConsejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, C1033AAJ, Ciudad de Buenos Aires, Argentina

^cDepartamento de Química (FCEyN), Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata, Argentina

ARTICLE INFO

Article history:

Received 8 August 2007

Accepted 9 April 2008

Available online 16 April 2008

Keywords:

phreatic water

Chasmagnathus granulatus

Neohelice granulata

nutrient flux

bioturbation

SW Atlantic estuaries

ABSTRACT

Along the coastal areas of the Southwest Atlantic estuaries and embayments, phreatic water often circulates through very extended areas (up to several hundred meters perpendicular to the coast), dominated by dense assemblages of deep burrows of the crab *Neohelice granulata* (formerly *Chasmagnathus granulatus*). This crab inhabits the intertidal area, from mudflats to marshes vegetated by species of *Spartina*, *Sarcocornia* and *Juncus*, generating extensive burrowing beds where burrow density may reach up to 60 burrows m⁻². Since the lower limit of the crab burrows is usually the water table, we investigated through field experiments the effect of *N. granulata* and their burrows on the chemical characteristics of this phreatic water. Water analysis from experimental (1) occupied burrows (with crabs), (2) unoccupied burrows (where crabs were excluded), and (3) sediment pore water show remarkable differences. Water oxygenation, and nitrate, ammonium and sulphate concentrations inside occupied burrows were higher than in the water inside unoccupied burrows or pore waters. Moreover, directed sampling of phreatic water entering and leaving the crab bed, shows that dissolved inorganic nitrogen concentration is enhanced as the water crosses the crab bed. These results may be ascribed to the fact that in the salt marsh the crabs spend most of their time within burrows, where presumably they store food (plants) and defecate. These activities generate an area of accumulation of excrements and nutrients in different decomposition states. The present work shows a novel way by which bioturbating organisms can affect nutrients exportation from salt marshes to the open waters.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Subterranean groundwater discharges may be an important fraction of the fresh water input in coastal areas without significant river input (Moore, 1996; Lambert and Burnett, 2003). In some cases, groundwater discharge into the sea may influence productivity, biomass, species composition and zonation by means of altered salinities, dissolved nutrient concentrations or dissolved pollutants (Johannes, 1980). Moreover, nutrient input by subterranean groundwater discharge plays an important role in nutrient cycling and primary productivity in the coastal ocean (Slomp and Van Cappellen, 2004). The input rates of nutrients can be significant due to nutrient concentrations in coastal groundwater which may be several orders of magnitude greater than those of the receiving coastal waters (Valiela et al., 1990). Nutrients in subterranean groundwater are mostly involved in geochemical transformations in their way through the bottom sediments (Slomp and Van Cappellen,

2004). Although it is known that groundwaters often pass through marsh and subtidal sediments before reaching the estuary (e.g. Harvey and Odum, 1990), the biological effects on their characteristics have not been taken into account previously.

Saltmarshes are usually the interface between land and water bodies, thus they directly interact with groundwater discharges (Harvey and Odum, 1990). Marshes are effective filters and nutrients transformers (e.g. Correl, 1981); their vegetation largely influences the sediment microbial activity by root excretion of organic substances and oxygen (e.g. Moriarty et al., 1986; Gribsholt and Kristensen, 2002). However, bioturbating organisms transport particles and fluid during feeding, burrowing, tube construction and irrigation activities (e.g. Aller, 1988; Aller and Aller, 1998; Fanjul et al., 2007). These organisms enhance water-sediment fluxes (Aller, 1988; Kristensen et al., 1991; Christensen et al., 2000), stimulating anaerobic degradation processes (Andersen and Kristensen, 1992; Banta et al., 1999; Fanjul et al., 2007), and also can influence the form and rate at which the metabolites are returned to the water column (Aller, 1983; Kristensen and Kostka, 2005). Thus, benthic macrofauna and flora may affect both the activity of microbial communities and transport processes in the sediment (Kristensen, 1984; Duarte, 1995; Fanjul et al., 2007).

* Corresponding author.

E-mail address: mefanjul@mdp.edu.ar (E. Fanjul).

Southwestern (SW) Atlantic estuaries and embayments (from southern Brazil, 23° S, to the northern Argentinean Patagonia, 41° S) are characterized by the presence of the burrowing crab, *Neohelice granulata* Dana (= *Chasmagnathus granulatus*; for recent taxonomic revision, see Sakai et al., 2006; see also Spivak et al., 1994; Iribarne et al., 1997). This crab inhabits the intertidal area, from mudflats to marshes vegetated by species of *Spartina*, *Sarcocornia* and *Juncus* (e.g. Botto et al., 2005; Bortolus, 2006), generating extensive burrowing beds (e.g. Iribarne et al., 1997; Botto et al., 2006). Burrows affect particle movement, working as passive traps of sediment organic matter (e.g. Botto et al., 2005, 2006), detritus (e.g. Iribarne et al., 2000; Botto et al., 2006) and polluting agents (e.g. Menone et al., 2004). Moreover, *N. granulata* has an important effect on particle redistribution (Botto and Iribarne, 2000; Fanjul et al., 2007), sediment chemistry (Botto et al., 2005; Gutiérrez et al., 2006; Fanjul et al., 2007) and drainage (Iribarne et al., 1997; Botto and Iribarne, 2000). Crab burrows are always open. In the mudflats burrows are short (up to 36 cm depth) with funnel-shaped entrances; however, in marshes they construct straight, long vertical tubular burrows up to 1 m depth (up to 40 mm diameter), reaching the water table (Iribarne et al., 1997; Bortolus and Iribarne, 1999). A certain amount of water is retained in burrows during low tide, and the presence of burrows with more than one aperture or burrows with small lateral tunnels inside may promote subterranean piping flow driven by a pressure difference above different openings during high tide (Allanson et al., 1992; Ridd, 1996; Perillo et al., 2005). Thus, groundwater seepage along tidal channels is favoured by the presence of burrows (Perillo et al., 2005). Phreatic reservoirs in SW Atlantic coastal marshes often circulate underneath very large areas drilled by crab burrows before discharging in the estuarine/coastal water (e.g. Iribarne et al., 1997). Furthermore, the burrows promote the contact between the atmosphere and the phreatic water affecting its redox state, while crabs activities directly impact over the chemical composition of the phreatic water.

Bioturbation has usually been considered as a diffusional process (i.e. continuous, symmetrical and over small distances; Boudreau, 1986; Boudreau et al., 1998), which stimulates nutrient benthic fluxes from intertidal sediments to coastal waters (Aller, 1988; Kristensen et al., 1991; Christensen et al., 2000). However, there is limited information on the potential effect of dense assemblages of animals that construct deep burrows (like *Neohelice granulata*). To our knowledge, no work has previously focused on the effect of bioturbation activities on subterranean groundwater discharges, even though these have been recognized as an important source of nutrients to coastal primary productivity (e.g. Johannes, 1980; Moore, 1999; Burnett et al., 2001). Given that in the marsh *N. granulata* burrows usually reach the phreatic reservoir (Bortolus and Iribarne, 1999), the chemical characteristics of the phreatic water may be modified via direct input of substances trapped and/or produced inside the burrows (i.e. crab excretions and metabolites of microbial activities), and also as a result of phreatic water oxygenation facilitated by the burrow structure. Using specifically targeted samplings and field experiments, we evaluate the effect of the burrowing crab, *N. granulata*, on the chemical characteristics of subterranean groundwater that discharges into coastal waters.

2. Materials and methods

2.1. Study site

The study was carried out between December 2005 and April 2006 at Mar Chiquita Coastal Lagoon (Argentina, 37°32' S, 57°19' W; Fig. 1), a shallow body of brackish water affected by low amplitude

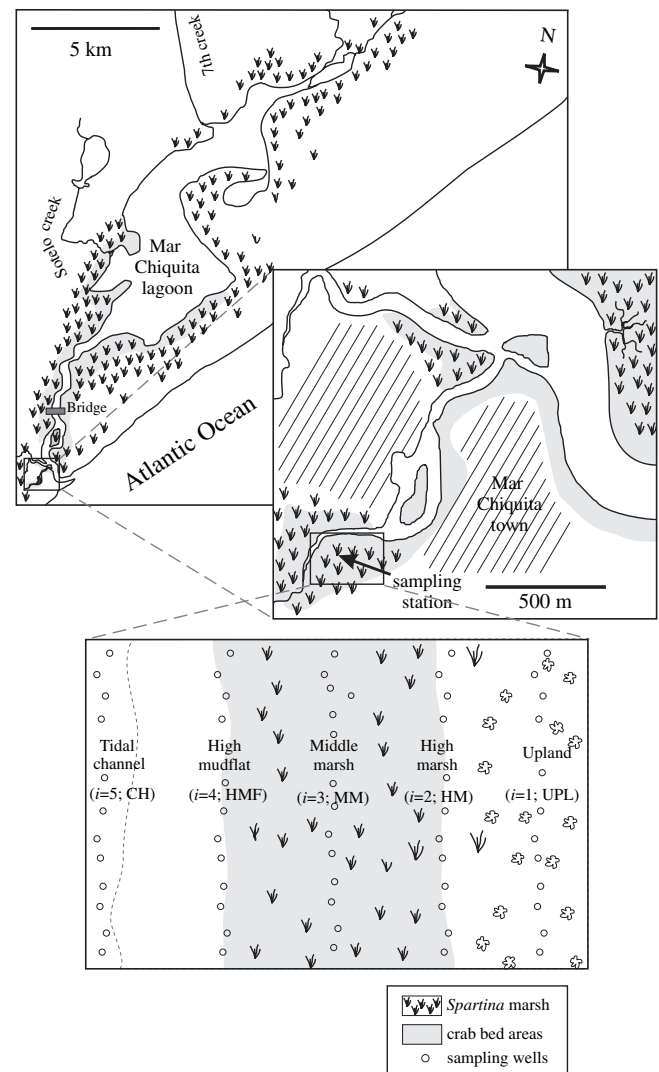


Fig. 1. Map of study area showing the spatial distribution of *Spartina densiflora* marshes and *Neohelice granulata* crab beds, and showing the location of sampling wells.

tides (<1.5 m) and characterized by soft bottoms (e.g. Fasano et al., 1982; Spivak et al., 1994; Iribarne et al., 1997). The lagoon receives a subterranean water contribution of around 0.8 hm³ yr⁻¹, which is approximately the 2.2% of its average volume (Fasano et al., 1982). Most of their intertidal sediments are characterized by the presence of the burrowing crab, *Neohelice granulata*, which generates extensive burrowing beds with up to 60 burrows m⁻² (e.g. Iribarne et al., 1997; Botto and Iribarne, 2000). In Mar Chiquita Coastal Lagoon, this crab inhabits the whole intertidal area from mudflats to vegetated marshes (e.g. Botto et al., 2005; Bortolus, 2006). Similar patterns occur in most of the intertidal areas of SW Atlantic estuaries and embayments (see Iribarne et al., 2005; Botto et al., 2006).

2.2. Mensurative experiment (comparison across habitat zones)

A directed field sampling was conducted in a marsh inhabited by *Neohelice granulata* (see Fig. 1) in order to evaluate if there is a change in the chemical characteristics of phreatic water as it crosses the crab bed. Wells were constructed using PVC pipes (to depths of 0–15 cm below the water table level, 10 cm diameter) to collect phreatic water at four different intertidal sites defined by their relative tidal height (see Figs. 1 and 2A) and the presence/absence of crabs across

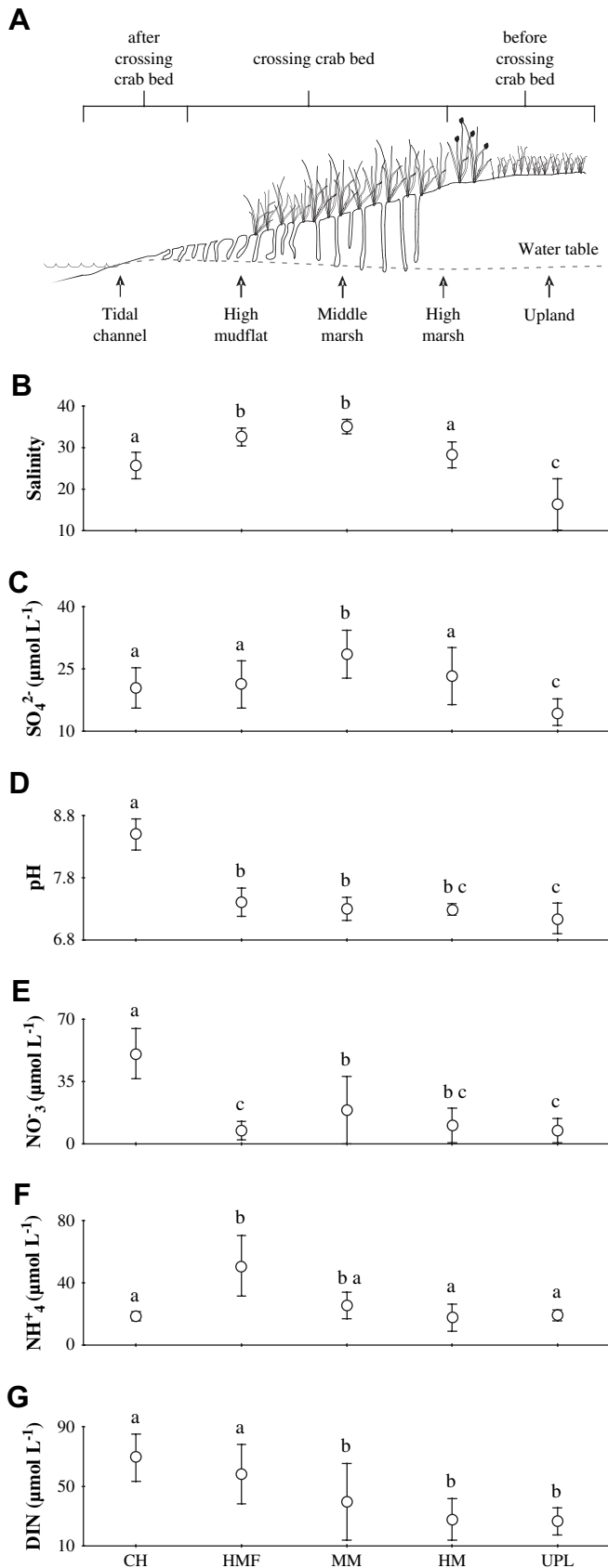


Fig. 2. (A) Typical cross section of *Neohelice granulata* bioturbated marsh. Chemical characteristics of phreatic water across the marsh area: (B) salinity; (C) sulphate concentration; (D) pH; (E) nitrate concentration; (F) ammonium concentration; (G) dissolved inorganic nitrogen concentration. Different letters represent significant differences between treatments (LSD test; $\alpha = 0.05$).

the marsh. Twenty-eight wells were constructed in two different lines parallel to the tidal level from inland of high marsh to collect phreatic water before crossing the crab bed (upland “UPL” and high marsh “HM” wells, see Figs. 1 and 2A). Fourteen wells (middle marsh, “MM”) were constructed in the middle marsh to collect phreatic water when crossing the crab bed; and 14 other identical wells were constructed in the high mudflat (“HMF”), near the marsh edge, to collect phreatic water after crossing the crab bed in the marsh (HMF wells, see Fig. 2A). Simultaneously with phreatic water sampling, we collected 14 water samples from the tidal channel (“CH”). Water samples for chemical analyses were collected during ebb tide periods, and immediately transported to the laboratory, filtered and preserved at $-20\text{ }^{\circ}\text{C}$ until analysis for salinity, sulphate, nitrate, ammonium, dissolved oxygen and pH. One-way ANOVA was performed on log-transformed data to test marsh zone effect on the chemical characteristics of phreatic water across the intertidal sites (Zar, 1999). Hereafter, *a posteriori* LSD tests were used when significant differences were found after ANOVA (Underwood, 1997).

Sediment salt content was measured at HMF, MM, HM and UPL site. Sediment samples ($n = 14$ for each site) were taken with a PVC core from each intertidal sites (3 cm diameter and 10 cm depth). Water content was obtained as the difference between wet and dry weight (after being dried at $60\text{ }^{\circ}\text{C}$ up to constant weight). Then, a subsample ($\sim 10\text{ g}$) was used to measure salinity of a 1:2 dry-sediment/distilled-water extract. Sediment salt content was calculated from water and salt sediment content data at each site. Porewater salinity could not be determined directly due to the low porewater content in these sediments. Differences in salt content across the intertidal sites were tested using one-way ANOVA (Zar, 1999).

Marsh sediments are most likely supersaturated in salts, and precipitated salts crystals on sediment surfaces are easily visible (E. Fanjul, personal observation). Given that during low tide phreatic water drains through sediments with high salt content, a progressive increase of phreatic water salinity by salt dissolution seems plausible. Assuming that the general movement of groundwater in the coastal zones is from upland sites to discharge sites in the tidal channels, we use a simple two-source mixing model described by the equation (1) to account for the observed salinity gradients.

$$PW_i^{sal} = a_i \times PW_{i-1}^{sal} \times \left(\frac{S_{i-1} + S_i}{2} \right) \times D_i + (1 - a_i) \times W_5^{sal} \quad (1)$$

In equation (1) i stands for the marsh site (the sites are numbered consecutively from $i = 1$ in UPL, to $i = 5$ in CH; see Fig. 1); PW_i^{sal} and S_i are the salinity and the sediment salt content measured at site i , respectively; W_5^{sal} is the salinity of channel water; and D_i is the normalized distance between i and $i - 1$ sites. The factors a_i and $(1 - a_i)$ represent the relative volume of water from UPL or CH sources, respectively, that are mixed at each site. Assuming that the volume of water from UPL and tidal channel that mixes at the i marsh site (i.e. a_i) is proportional to the distance between i site and the two sources (i.e. UPL and CH), the model allow us to estimate the salinities of phreatic water at HM, MM, and HMF sites. Data of PW_i^{sal} and S_i were resampled independently ($n = 100$) for each marsh site (Manly, 1998), in order to estimate the salinities at different sites from equation (1). The null hypothesis of no difference between measured and estimated salinities of phreatic water at HM, MM, and HMF sites were evaluated independently for each site using t -test (Zar, 1999); the degrees of freedom used in this t -test were those of the original data following a conservative approach (Manly, 1998).

2.3. Manipulative experiments testing effects of crabs and burrows

A manipulative field experiment was conducted to evaluate if there is an effect of *Neohelice granulata* activities on the quantity

and quality of dissolved inorganic nutrients in the water inside the burrows. The experiment was deployed in the middle level of a *Spartina*-marsh (MM level, see Fig. 1). To distinguish if changes in the quality of burrow water could be simply explained by the increment of exchange area by burrow presence, or caused by the added effect of crab activities inside the burrow (i.e. bioturbation activities, feeding, excretions), three experimental treatments were employed: (1) areas with artificial burrows and crabs (burrow + crab treatment; thereafter B + C); (2) areas with artificial burrows but without crabs (burrow treatment; thereafter B); and (3) crab exclusions areas used as “control” treatment without effect of crabs or burrows (NBC). One adult crab was added per burrow at B + C treatment. Each experimental plot was separated at least 5 meters from each other, and was surrounded with plastic mesh boxes (opening 0.75 cm) of 1 m side and 0.4 m tall ($n = 8$ per treatment). Treatments were randomly assigned to each plot, and all experimental plots were placed on areas initially not occupied by crabs or burrows to evaluate their actual effects on the sediment chemistry. Artificially-constructed burrows (1.2 m depth; 30 burrows m^{-2}) were created by pushing a PVC pipe (3 cm diameter) into the sediment and then the pipe and the sediment inside were removed (see Iribarne et al., 2005). Boxes were periodically (i.e. by-weekly) observed to make sure that the crabs were alive and active in B + C plots, and that they did not colonize the control plots (when a crab was found in these plots, it was removed by hand). In this experiment, burrow water collection was conducted after 4 months to ensure minimum sediment disturbance after the set up. In treatments B + C and B, burrow water was collected by means of a 60 ml syringe attached to a 5 mm acrylic tube gently immersed 15 cm into the burrow. Water samples of treatment NBC were extracted using wells (15 cm depth) constructed using PVC pipes (30 mm diameter). These wells allowed us to collect vertically composite pore waters. All water samples were collected during ebb tide periods and immediately transported to the laboratory, filtered and conserved at $-20\text{ }^{\circ}\text{C}$ until analysis. The null hypothesis of no differences in the dissolved inorganic nutrients of burrow water (see below) between different treatments was evaluated by one-way ANOVAs (Zar, 1999).

2.4. Chemical analyses

Salinity, sulphate (SO_4^{2-}), nitrate (NO_3^-), ammonium (NH_4^+), dissolved oxygen (O_2) and pH were measured in the phreatic and burrow water. Salinity was measured using a handheld refractometer. Sulphate and nitrate were analyzed after the elimination of Cl^- by ion-chromatography (Universal Anion[®] column 150 mm, $\text{HCO}_3^-/\text{CO}_3^{2-}$ mobile phase 1.25/0.85 mmol L^{-1} , suppressed conductivity detection). NH_4^+ was measured following the blue-indophenol method (Solórzano, 1969). Dissolved oxygen was measured in situ using an Oakton[®] Waterproof DO 300 Meter. pH was measured in situ using a combination pH electrode (HI 1230B, Hanna Instruments).

3. Results

3.1. Mensurative experiment (comparison across habitat zones)

Solute concentrations in phreatic water showed differences between sites. Salinity was twice as low in the upland sites than in middle marsh sampling station (UPL: mean = 16, SD = 6, $n = 14$; MM: mean = 35, SD = 1, $n = 14$), and then decreased through the high mudflat (HMF) sites to reach intermediate values on the tidal channel water (CH: mean = 26, SD = 3, $n = 14$; ANOVA: $F_{4,65} = 40.39$, $p < 0.001$; Fig. 2B). Sulphate concentration was highest at the MM sites, and showed the lowest value in the upland

sites (log transformed data: $F_{4,65} = 12.45$, $p < 0.001$; Fig. 2C). The pH of phreatic water was lower before (i.e. upland) than after the crab bed (i.e. HMF, see Fig. 1) showing intermediate values in HM and MM sites (log transformed data: $F_{4,65} = 88.4$, $p < 0.001$; Fig. 2D). Nitrate concentration on phreatic water increased when approaching from high elevations to MM sites, showing the lowest values in HMF sites and the highest value in the tidal channel water (log transformed data: $F_{4,65} = 20.31$, $p < 0.001$; Fig. 2E). Ammonium content was in average 2.5 times higher in the HMF than in the others sampling sites (log transformed data: $F_{4,65} = 15.70$, $p < 0.001$; Fig. 2F). DIN was higher at CH and HMF sites than at upland, high and middle marsh sites (log transformed data: $F_{4,65} = 14.33$, $p < 0.001$; Fig. 2G).

Sediment salt content was higher at middle marsh than at high plain, high marsh and upland sites. Salt sediment content at upland sites was 14 times smaller than at the other sites ($F_{3, 36} = 38.28$, $p < 0.001$). No differences were found between measured and model-estimated values of phreatic water salinity at HM (measured: mean = 28, SD = 3; model-estimated: mean = 24, SD = 9; $t = 1.71$, $df = 26$, $p = 0.100$), MM (measured: mean = 35, SD = 2; model-estimated: mean = 37, SD = 10; $t = -0.74$, $df = 26$, $p = 0.468$) and HMF sites (measured: mean = 33, SD = 2; model-estimated: mean = 32, SD = 5; $t = 0.27$, $df = 26$, $p = 0.789$). Thus, the mixing model accurately describes the gradient in water salinity of phreatic water across the marsh sites, indicating that groundwater flows from upland sites to the tidal channel, dissolving precipitated salts of marsh sediments.

3.2. Manipulative experiments testing effects of crabs and burrows

Experimental manipulations showed that *Neohelice granulata* presence affected burrow water quality. Sulphate concentration was lower in burrow water in B treatment and in porewater in NBC treatment, than in burrow water in B + C treatment ($F_{2,21} = 9.85$, $p < 0.05$; Fig. 3B). Dissolved oxygen was higher in burrow water of the B + C treatment than other treatments, and dissolved oxygen in water of unoccupied burrows of the B treatment was higher than in the NBC treatment (square-root transformed data; $F_{2,21} = 725.01$, $p < 0.05$; Fig. 3C). Salinity, pH, ammonium concentration, nitrate concentration and DIN concentration in water inside burrows in the B + C treatment were higher than in porewaters in non-bioturbated NBC treatment (salinity: $F_{2,21} = 4.31$, $p < 0.05$, Fig. 3A; pH: $F_{2,21} = 11.00$, $p < 0.05$, Fig. 3D; ammonium: $F_{2,21} = 9.73$, $p < 0.05$, Fig. 3E; nitrate: $F_{2,21} = 6.52$, $p < 0.05$, Fig. 3F; DIN: $F_{2,21} = 10.72$, $p < 0.05$, Fig. 3G). For all these variables, the water inside unoccupied burrows (i.e. B treatment) presented intermediate values when compared with the B + C and NBC treatments (Fig. 3A,D–G).

4. Discussion

Our observations and experiments showed that phreatic water increase their DIN content as it crosses the crab beds of *Neohelice granulata*. Also, the manipulative field experiment revealed that crab activities modify water quality, since the amount of dissolved substances in water burrows was always higher than that of porewater from non-bioturbated sediments, while water inside unoccupied burrows show intermediate quantities of dissolved nutrients.

4.1. Phreatic water circulation underneath crab beds

Changes in salinity of the phreatic water across marsh zones confirm that groundwater flows from inland to tidal channels. Our data shows horizontal salinity patterns typical of a “subterranean estuary” (sensu Moore, 1999; Fig. 2B) in which fresh and saltwater are mixed. At low tide, the tidal channel contains a blend of

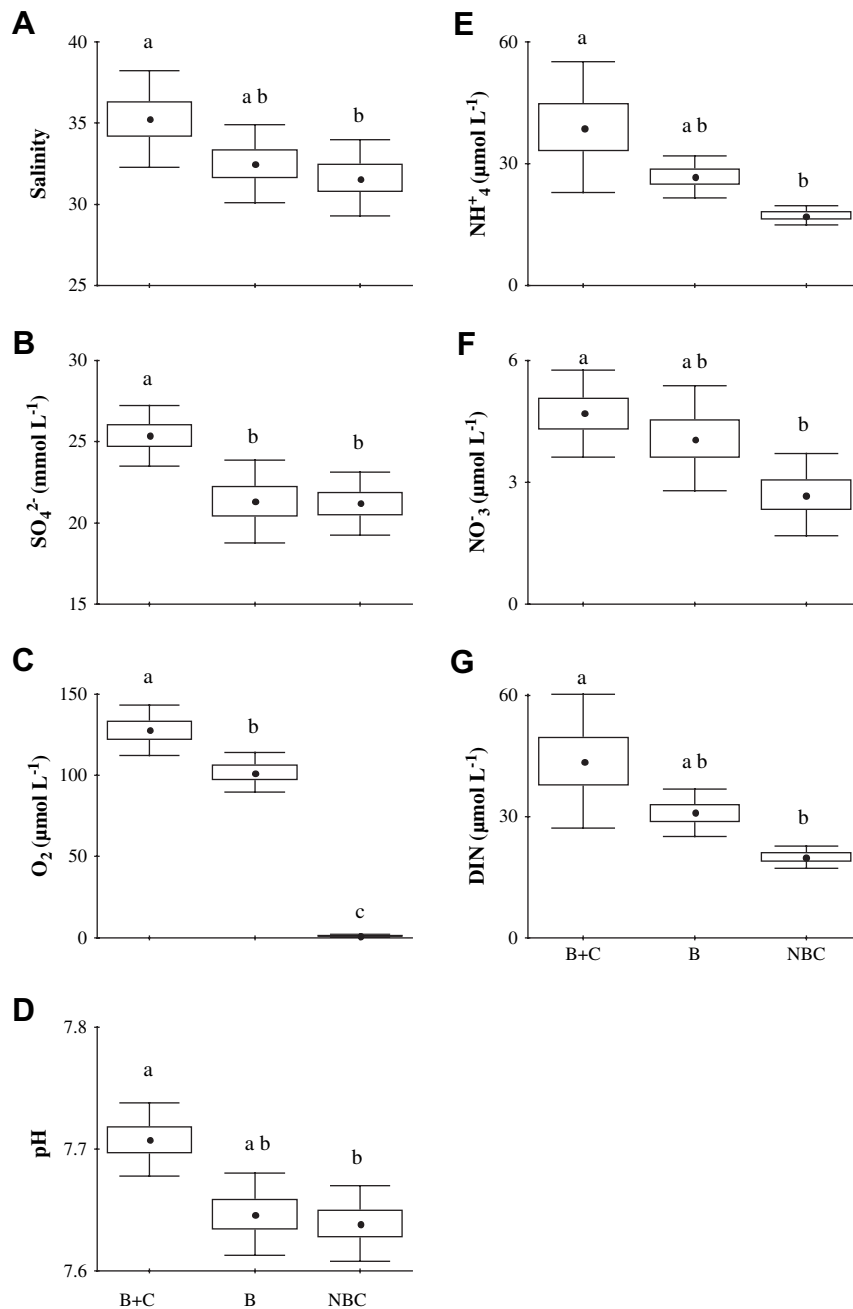


Fig. 3. Burrow water (A) salinity; (B) sulphate concentration; (C) dissolved oxygen concentration; (D) pH; (E) ammonium concentration; (F) nitrate concentration; (G) dissolved inorganic nitrogen content at B + C, B and NBC experimental treatments. Different letters represent significant differences between treatments (LSD test; $\alpha = 0.05$).

seawater and surface freshwater derived from inland areas. In our study site, salinity of tidal channel was lower than salinity from the phreatic water below the marsh sediment. Much of the groundwater that is discharged directly to estuaries is intercepted by marshes, and the groundwater is then a component of the marsh pore water subject to desiccation (Harvey and Odum, 1990). Moreover, it has been shown that salt excluded at the mangrove roots can cause the salt concentration in the surrounding sediments to rise above 50 kg m^{-3} (Passioura et al., 1992). Thus, in our study site, phreatic water drains through sediments with high salt concentration possibly due to both desiccations of high quantities of seawater accumulated in the crab burrows during low tide, and salt excretions by *Spartina* roots. The measured salinities of phreatic water across marsh sites were almost equal to the model-estimated value for each site, indicating that the two-source mixing model

accurately describes the gradient in water salinity of phreatic water across the marsh. Thus, the phreatic water salinity pattern found in the study site can result from dissolution of salt from sediments by groundwater, as it flows through marsh sediments from inland to tidal channel, and also as a result of mixing with tidal channel water.

The increase in nitrate concentration of phreatic water from the high to the middle marsh zones could be due to crab burrows (Fig. 2E), because water inside both occupied and unoccupied burrows is richer in nitrate (see Fig. 3F). In the marsh areas, high quantities of nitrate may enter the phreatic water via crab burrows, which act as a conduit for dissolved substances (Stieglitz et al., 2000). Thus, in this area, crabs enhance nitrate content of phreatic water (see Section 3). In this area, the removal of nitrate through denitrification (and nitrate reduction) could be limited by the high

redox state of sediments produced by the low inundation frequency of higher marsh zones, since denitrifying bacteria consume nitrate under anoxic conditions (Herbert, 1999). The depletion in nitrate from middle marsh toward the mudflat may be the result of biological consumption of nitrate by plant uptake or by denitrification coupled to organic matter degradation. This last process, which occurs in anoxic sediments (see Herbert, 1999), is stimulated by crab activities (Fanjul et al., 2007). Therefore, both plant assimilation and denitrification may act as sinks of the high nitrate content of phreatic and burrow water. Water inside burrows is also enhanced in ammonium (see Fig. 3E), possibly due to both ammonium accumulation from crab excretions and the high rates of organic matter degradation (that produce ammonium as end N product; Herbert, 1999). As a result, phreatic water becomes progressively more concentrated in ammonium along the direction of groundwater flow. When this water is further mixed with oxic water from the tidal channel, part of the ammonium may be removed through nitrification and converted to nitrate (see Slomp and Van Cappellen, 2004). Considering that the individual absolute values of nitrate and ammonium concentration may be influenced by water evaporation or mixing, we verify that the ratio between nitrate-ammonium and total DIN, which are free from this source of error, followed the same trend as nitrate and ammonium concentration patterns. Thus, we can safely conclude that groundwater nutrients are mostly subject to active biogeochemical transformations.

4.2. Burrow water enhancement in inorganic nutrients

Given that marshes are located between land and sea, and due to their relatively high rates of biogeochemical transformations (Alongi, 1998), many works suggest that marshes actually serve as nutrient filters intercepting and absorbing land-derived nutrients, thereby reducing nutrient input to estuarine and coastal waters (Nixon, 1980; Correl, 1981; Valiela et al., 2000). Our results show that bioturbating organisms may affect this filtering of land-derived nutrients, since they perforate the marsh sediments influencing the nutrients that leave the marsh through phreatic water. Moreover, in the salt marsh, crabs spend most of their time within their burrows, where they defecate and store food (plants and detritus). This process generates the accumulation of excrement and nutrients in different decomposition states within their burrows, which leave the marsh through phreatic water.

The coupling between benthic and pelagic systems is a critical component of the nutrient cycling within estuarine systems and supporting estuarine food web (e.g. Nixon, 1980). This coupling consists of deposition and subsequent remineralization of organic matter (with the release of inorganic nutrients) and transport of dissolved nutrients back into the overlying water column mostly by benthic fluxes (Jahnke et al., 2003). Several factors control organic matter degradation in intertidal sediments, including organic matter chemical composition, decomposition stage, adsorption to mineral surfaces, bioturbation and environmental conditions such as temperature and electron acceptor availability (e.g. Keil et al., 1994; Fenichel et al., 1998). In SW Atlantic intertidal sediments bioturbated by *Neohelice granulata*, the high rates of organic matter degradation (Fanjul et al., 2007) produce high quantities of remineralized nutrients that are accumulated into the burrows (our results).

4.3. Marsh filters function affected by crab burrowing

Neohelice granulata generates extensive burrowing beds (e.g. Iribarne et al., 1997; Botto et al., 2006). While constructing and maintaining their burrows, crabs excavate large quantities of sediment (up to $6 \text{ kg m}^{-2} \text{ d}^{-1}$) which is deposited on the surface (Iribarne et al., 1997). This process generates a high value of the

bioturbation coefficient ($250 \text{ cm}^2 \text{ yr}^{-1}$; sensu Wheatcroft, 1992; Fanjul et al., 2007). Moreover, in marshes crabs construct straight, long vertical tubular burrows up to 1 m depth (up to 40 mm diameter; Iribarne et al., 1997), that directly (bioturbation) and indirectly (burrow structures) enhance sediment oxygenation (Daleo et al., 2007; Fanjul et al., 2007). Also, direct measurements of chemical profiles across *N. granulata* burrow-sediment interface; indicate that oxic wall thickness (i.e. the radial distance from the burrow wall in which oxygen concentration reaches the radially averaged concentration for bulk sediment at a given depth) at 5 cm depth is 0.875 cm (E. Fanjul, personal observation). Thus, the large changes on sediment physical characteristics generated by this crab affect the rates and pathways of organic matter degradation (Fanjul et al., 2007), and are expected to affect microbial processes and nutrients flows to adjacent systems.

The marsh sediment has generally low permeability, due to very high clay content (e.g. Botto and Iribarne, 2000). However, it is known that burrows and galleries may affect sediment permeability (Meadows and Meadows, 1991) providing an efficient system for transport of water and solutes to and from the substrate (Stieglitz et al., 2000). Burrows are real conduits for drainage increasing sediment permeability (Jones and Jago, 1993; Stieglitz et al., 2000), while binding substances produced by many burrowing invertebrates may clog the interstitial spaces of the between-burrow sediment (Frankel and Mead, 1973). At SW Atlantic intertidal sediments, *Neohelice granulata* increase sediment porosity (ϕ) both at surface and depth (mean $\phi = 0.555$ at bioturbated sediments and $\phi = 0.435$ at non-bioturbated sediments; E. Fanjul, personal observation). Since *N. granulata* does not secrete mucus, but constructs deep and large burrows, it is reasonable to assume that this crab increases the sediment permeability. Besides the change in the permeability of bioturbated sediments, and the enhancement remineralization of nutrients induced by crab activities and burrow structures (Fanjul et al., 2007), high quantities of nutrients may enter the phreatic reservoir via crab burrows. In this way, transport of dissolved nutrients back into the estuarine water column via phreatic water is enhanced by crab activities and burrow presence. Considering the extensive local and geographic spatial distribution of the *N. granulata* crab beds (see Iribarne et al., 2005; Botto et al., 2006), their high densities and the large size of their burrows (Iribarne et al., 1997; Botto and Iribarne, 2000), we anticipate important consequences on nutrient fluxes at large scale. Thus, our results show a novel form by which bioturbating organisms can affect quantitative and qualitatively the export of nutrients from salt marshes to the open estuarine/coastal waters. Based on those results, we believe that other species that construct semi-permanent deep burrows in marshes such as those formed by other crab species (e.g. Letzsch and Frey, 1980; Bertness, 1985) or ghost shrimps (e.g. Posey, 1986; Posey et al., 1991) may play a role similar to *N. granulata*.

Acknowledgments

We thank P. Daleo and M. Escapa for field help. This project was supported by Universidad Nacional de Mar del Plata, Fundación Antorchas (Grant # 13900-13), ANPCYT (1-7213) and CONICET (PIP 2851; 165-01), all granted to O.I. E.F. and A.C. were supported by scholarships from CONICET. This work is part of the doctoral thesis of E.F. at the UNMdP.

References

- Allanson, B.R., Skinner, D., Imberger, J., 1992. Flow in prawn burrows. Estuarine, Coastal and Shelf Science 35, 253–266.
- Aller, R.C., 1983. The importance of the diffusive permeability of animal burrow linings in determining marine sediment chemistry. Journal of Marine Research 41, 299–322.

- Aller, R.C., 1988. Benthic fauna and biogeochemical processes in marine sediments: the role of burrow structures. In: Blackburn, T.H., Sørensen, J. (Eds.), Nitrogen Cycling in Coastal Marine Environments. John Wiley, New York, pp. 301–338.
- Aller, R.C., Aller, J.Y., 1998. The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. *Journal of Marine Research* 56, 905–936.
- Alongi, D.M., 1998. Coastal Ecosystem Processes. CRC Press, New York, 419 pp.
- Andersen, F.Ø., Kristensen, E., 1992. The importance of benthic macrofauna in decomposition of microalgae in a coastal marine sediment. *Limnology and Oceanography* 37, 1392–1403.
- Banta, G.T., Holmer, M., Jensen, M.H., Kristensen, E., 1999. Effects of two polychaete worms, *Nereis diversicolor* and *Arenicola marina*, on aerobic and anaerobic decomposition in sandy marine sediment. *Aquatic Microbial Ecology* 19, 189–204.
- Bertness, M.D., 1985. Fiddler crab regulation of *Spartina alterniflora* production on a New England salt marsh. *Ecology* 66, 1042–1055.
- Bortolus, A., 2006. The austral cordgrass *Spartina densiflora* Brong. : its taxonomy, biogeography and natural history. *Journal of Biogeography* 33, 158–168.
- Bortolus, A., Iribarne, O., 1999. Effects of the SW Atlantic burrowing crab *Chasmagnathus granulata* on a *Spartina* salt marsh. *Marine Ecology Progress Series* 178, 79–88.
- Botto, F., Iribarne, O., 2000. Contrasting effects of two burrowing crabs (*Chasmagnathus granulata* and *Uca uruguayensis*) on sediment composition and transport in estuarine environments. *Estuarine, Coastal and Shelf Science* 51, 141–151.
- Botto, F., Valiela, I., Iribarne, O., Martinetto, P., Alberti, J., 2005. Impact of burrowing crabs on C and N sources, control, and transformations in sediments and food webs of SW Atlantic estuaries. *Marine Ecology Progress Series* 293, 155–164.
- Botto, F., Iribarne, O., Gutiérrez, J., Bava, J., Gagliardini, A., Valiela, I., 2006. Ecological importance of passive deposition of organic matter into burrows of the SW Atlantic crab *Chasmagnathus granulatus*. *Marine Ecology Progress Series* 312, 201–210.
- Boudreau, B.P., 1986. Mathematics of tracer mixing in sediments. II. Non local mixing and biological conveyor-belt phenomena. *American Journal of Science* 286, 199–238.
- Boudreau, B.P., Mucci, A., Sundby, B., Luther, G.W., Silverberg, N., 1998. Comparative diagenesis at three sites on the Canadian continental margin. *Journal of Marine Research* 56, 1259–1284.
- Burnett, W.C., Taniguchi, M., Oberdorfer, J., 2001. Measurement and significance of the direct discharge of groundwater into the coastal zone. *Journal of Sea Research* 46, 109–116.
- Correl, D.L., 1981. Nutrient mass balances for the watershed, headwaters intertidal zones, and basin of the Rhode River estuary. *Limnology and Oceanography* 26, 1142–1149.
- Christensen, B., Vedel, A., Kristensen, E., 2000. Carbon and nitrogen fluxes in sediment inhabited by suspension-feeding (*Nereis diversicolor*) and non-suspension-feeding (*N. virens*) polychaetes. *Marine Ecology Progress Series* 192, 203–217.
- Daleo, P., Fanjul, E., Mendez Casariego, A., Silliman, B.R., Bertness, M.D., Iribarne, O., 2007. Ecosystem engineers activate mycorrhizal mutualism in salt marshes. *Ecology Letters* 10, 902–908.
- Duarte, C., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41, 87–112.
- Fanjul, E., Grela, M.A., Iribarne, O., 2007. Effects of the dominant SW Atlantic intertidal burrowing crab *Chasmagnathus granulatus* on sediment chemistry and nutrient distribution. *Marine Ecology Progress Series* 341, 177–190.
- Fasano, J.L., Hernandez, M.A., Isla, F.I., Schnack, E.J., 1982. Aspectos evolutivos y ambientales de la laguna Mar Chiquita (provincia de Buenos Aires, Argentina). *Oceanologica Acta No. Spec.* 285–292.
- Fenchel, T., King, G.M., Blackburn, T.H., 1998. Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling. Academic Press, London, 307 pp.
- Frankel, L., Mead, D.J., 1973. Mucilaginous matrix of some estuarine sands in Connecticut. *Journal of Sedimentary Petrology* 43, 1090–1095.
- Gribsholt, B., Kristensen, E., 2002. Effects of bioturbation and plant roots on salt marsh biogeochemistry: a mesocosm study. *Marine Ecology Progress Series* 241, 71–87.
- Gutiérrez, J.L., Jones, C.G., Groffman, P.M., Findlay, S.E.G., Iribarne, O.O., Ribeiro, P.D., Bruschetti, C.M., 2006. The contribution of crab burrow excavation to carbon availability in surficial salt-marsh sediments. *Ecosystems* 9, 647–658.
- Harvey, J.W., Odum, W.E., 1990. The Influence of Tidal Marshes on Upland Groundwater Discharge to Estuaries. *Biogeochemistry* 10, 217–236.
- Herbert, R.A., 1999. Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiology Reviews* 23, 563–590.
- Iribarne, O., Bortolus, A., Botto, F., 1997. Between-habitats differences in burrow characteristics and trophic modes in the south western Atlantic burrowing crab *Chasmagnathus granulata*. *Marine Ecology Progress Series* 155, 132–145.
- Iribarne, O., Botto, F., Martinetto, P., Gutiérrez, J., 2000. The role of the SW Atlantic burrowing crab in sequestering debris. *Marine Pollution Bulletin* 40, 1057–1062.
- Iribarne, O., Bruschetti, M., Escapa, M., Bava, J., Botto, F., Gutierrez, J., Palomo, G., Delhey, K., Petracchi, P., Gagliardini, A., 2005. Small- and large-scale effect of the SW Atlantic burrowing crab *Chasmagnathus granulatus* on habitat use by migratory shorebirds. *Journal of Experimental Marine Biology and Ecology* 315, 87–101.
- Jahnke, R.A., Alexander, C.R., Kostka, J.E., 2003. Advective pore water input of nutrients to the Satilla River Estuary, Georgia, USA. *Estuarine, Coastal and Shelf Science* 56, 641–653.
- Johannes, R.E., 1980. The ecological significance of the submarine discharge of groundwater. *Marine Ecology Progress Series* 3, 365–373.
- Jones, S.E., Jago, C.F., 1993. In situ assessment of modification of sediment properties by burrowing invertebrates. *Marine Biology* 115, 133–142.
- Keil, R., Martlucan, D., Prahl, F., Hedges, J., 1994. Sorptive preservation of labile organic matter in marine sediments. *Nature* 370, 549–552.
- Kristensen, E., 1984. Effects of natural concentrations on nutrient exchange between a polychaete burrow in estuarine sediment and the overlying water. *Journal of Experimental Marine Biology and Ecology* 75, 171–190.
- Kristensen, E., Kostka, J.E., 2005. Macrofaunal burrows and irrigation in marine sediment: microbiological and biogeochemical interactions. In: Kristensen, E., Haese, R.R., Kostka, J.E. (Eds.), Interactions between macro- and micro-organisms in marine sediments. Coastal and Estuarine Studies 60. American Geophysical Union, Washington, DC, pp. 125–157.
- Kristensen, E., Jensen, M.H., Aller, R.C., 1991. Direct measurement of dissolved inorganic nitrogen exchange and denitrification in individuals polychaete (*Nereis virens*) burrows. *Journal of Marine Research* 49, 355–377.
- Lambert, M.J., Burnett, W.C., 2003. Submarine groundwater discharge estimates at a Florida coastal site based on continuous radon measurements. *Biogeochemistry* 66, 55–73.
- Letzsch, W.S., Frey, R.W., 1980. Erosion of salt marsh tidal banks, Sapelo Island, Georgia. *Senckenbergiana Maritima* 12, 201–212.
- Manly, B.F., 1998. Randomization, bootstrap and Monte Carlo methods in biology. Chapman and Hall, New York, 424 pp.
- Meadows, P.S., Meadows, A., 1991. The geotechnical and geochemical implications of bioturbation in marine sedimentary ecosystems. In: Meadows, P.S., Meadows, A. (Eds.), The Environmental Impact of Burrowing Animals and Animal Burrows. Symposium of the Zoological Society of London, London, pp. 157–181.
- Menone, M., Miglioranza, K.S.B., Iribarne, O., Aizpún de Moreno, J.E.A., Moreno, V.J., 2004. The role of burrowing beds and burrows of the SW Atlantic intertidal crab *Chasmagnathus granulata* in trapping organochlorine pesticides. *Marine Pollution Bulletin* 48, 240–247.
- Moore, W.S., 1996. Large groundwater inputs to coastal waters revealed by Ra-226 enrichments. *Nature* 380, 612–614.
- Moore, W.S., 1999. The subterranean estuary: a reaction zone of ground water and sea water. *Marine Chemistry* 65, 111–125.
- Moriarty, D.L.W., Iverson, R.L., Pollard, P.C., 1986. Exudation of organic carbon by the seagrass *Halodule wrightii* Aschers and its effect on bacterial growth in the sediment. *Journal of Experimental Marine Biology and Ecology* 96, 115–126.
- Nixon, S.W., 1980. Between coastal marshes and coastal waters—a review of twenty years of speculation and research on the role of salt marshes ad coastal waters in estuarine productivity and water chemistry. In: Hamilton, P., McDonald, K.B. (Eds.), Estuarine and Wetland Processes with Emphasis on Modeling. Plenum Press, New York, pp. 437–526.
- Passioura, J.B., Ball, M.C., Knight, J.H., 1992. Mangroves may salinize the soil and in doing so limit their transpiration rate. *Functional Ecology* 6, 476–481.
- Perillo, G.M.E., Minkoff, D., Piccolo, M.C., 2005. Novel mechanism of stream formation in coastal wetlands by crab-fish-groundwater interaction. *Geo-Marine Letters* 25, 217–220.
- Posey, M.H., 1986. Changes in a benthic community associated with dense beds of a burrowing deposit-feeder, *Callinassa californiensis*. *Marine Ecology Progress Series* 31, 15–22.
- Posey, M.H., Dumbauld, B.R., Armstrong, D.A., 1991. Effects of a burrowing mud shrimp, *Upogebia pugettensis* (Dana), on abundances of macro-infauna. *Journal of Experimental Marine Biology and Ecology* 148, 283–294.
- Ridd, P.V., 1996. Flow through animal burrows in mangrove swamps. *Estuarine, Coastal and Shelf Science* 43, 617–625.
- Sakai, K., Türkay, M., Yang, S.-L., 2006. Revision of the Helice/Chasmagnathus complex (Crustacea: Decapoda: Brachyura). *Abhandlungen Der Senckenbergischen Naturforschenden Gesellschaft* 565, 1–76.
- Slomp, C.P., Van Cappellen, P., 2004. Nutrient inputs to the coastal ocean through submarine groundwater discharge: controls and potential impact. *Journal of Hydrology* 295, 64–86.
- Solórzano, L., 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnology and Oceanography* 14, 799–801.
- Spivak, E.D., Anger, K., Luppi, T., Bas, C., Ismael, D., 1994. Distribution and habitat preferences of two grapsid crab species in Mar Chiquita Lagoon (Province of Buenos Aires, Argentina). *Helgoländer Meeresunters* 48, 59–78.
- Stieglitz, T., Ridd, P., Müller, P., 2000. Passive irrigation and functional morphology of crustacean burrows in a tropical mangrove swamp. *Hydrobiologia* 421, 69–76.
- Underwood, A.J., 1997. Experiments in Ecology: their Logical Design and Interpretation using Analysis of Variance. Cambridge University Press, Cambridge, 504 pp.
- Valiela, I., Costa, J., Foreman, K., Teal, J.M., Howes, B., Aubrey, D., 1990. Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters. *Biogeochemistry* 10, 177–197.
- Valiela, I., Cole, M.L., McClelland, J., Hauxwell, J., Cebrian, J., Joye, S.B., 2000. Role of salt marshes as part of coastal landscapes. In: Weinstein, M.P., Kreeger, D.A. (Eds.), Concepts and Controversies of Tidal Marsh Ecology. Kluwer Academic Publishers, Dordrecht, pp. 23–38.
- Wheatcroft, R.A., 1992. Experimental test for particle size-dependent bioturbation in the deep ocean. *Limnology and Oceanography* 37, 90–104.
- Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall, New Jersey, 663 pp.