

Crab-mediated phenotypic changes in *Spartina densiflora* Brong.

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Abstract

Although plant phenotypic plasticity has been historically studied as an important adaptive strategy to overcome herbivory and environmental heterogeneity, there are several aspects of its ecological importance that remain controversial. The burrowing crab *Chasmagnathus granulata* eats *Spartina densiflora*, and also causes several geomorphologic changes that indirectly affect *Spartina* growth. Here we evaluate if this crab affects the sexual reproductive effort of *S. densiflora* by mediating changes in plant phenotypic plasticity (i.e., shape of leaves and spikes) while affecting aboveground production, and if these effects interact with disturbance intensity. We conducted local and regional surveys and two-year field experiments manipulating the density of crabs in a mature *Spartina* marsh where we clipped at ground level different 1 × 1 m marsh areas to create and compare crab's effect on young (plants growing after the clipping) and mature (unclipped) *Spartina* stands. Our results suggest that crabs mediate the phenotypic plasticity of sexual reproductive structures of *Spartina*. Crabs induced an increase in seed production (up to 721%) and seed viability, potentially favoring *Spartina* dispersal and colonization of distant sites. This effect appears to be maximal when combined with the experimental clipping disturbance. Crabs also exerted a strong effect on clipped plants by increasing the number of standing dead stems and decreasing the photosynthetic area and leaf production. These effects disappear in about two years if no other disturbance occurs. An a posteriori regional field survey agreed with our experimental results corroborating the prediction that plants in old undisturbed marshes have lower sexual reproductive effort than plants in highly disturbed marshes populated by burrowing-herbivore crabs. All these phenotypic changes have important taxonomic and macro-ecological implications that should not be ignored in discussions of applied ecology and environmental management.

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1. Introduction

Plants are able to respond to environmental changes through developmental plasticity in many traits (Sultan, 2000). Some plants can vary their phenotype, optimizing the use of resources and increasing their success within a given community (Callaway et al., 2003). However, when sexual reproductive structures also have a plastic expression, environmental changes may not only affect plant survival but also plant reproductive success and dispersal. For example, herbivory may regulate the magnitude of the production of flowers, spikes and seeds

(Paige and Whitham, 1987; Howe and Westley, 1988), and similar effects are known to be caused by herbivores' secondary activities (e.g., burrowing or walking; Crawley, 1983; Hik et al., 1992). By increasing the production and dispersion of seeds, a given plant–animal interaction may increase the chances for the plant species to colonize and invade distant areas where the same plant–animal association may not exist (Bortolus, 2001). Therefore, a plant–animal interaction present at a given site may affect the structure and dynamics of a community in a different and distant site. This long-distance interaction has been historically overlooked by community ecologists, for they typically focus on species that are (or were) present in the studied community. In this paper we show evidence supporting the idea that ecological interactions may have important long-distance effects that should be

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incorporated in discussions of community ecology and environmental management.

The sexual reproductive strategy is not as common in clonal plants as it is in non-clonal plants (Harper, 1977; Silander, 1979). *Spartina* seeds are usually mentioned as having a poor viability (i.e., low proportion of viable seeds), and the massive clones presented in most species of this genus are believed to determine their success and dominance in marsh habitats (Adam, 1993; Pennings and Bertness, 2001). However, because sexual reproduction was not historically recognized as an important propagation mechanism for *Spartina* species (Mobberley, 1956; Adam, 1993; Mitsch and Gosselink, 2000; Pennings and Bertness, 2001), its importance may have been long overlooked. Although the southern cordgrass *Spartina densiflora* Brongniart (1829) is the only species of the genus native and commonly found dominating the salt marshes of southern South America (Costa and Davy, 1992; Bortolus, 2001), few papers have focused on its biology and ecology. This species is able to colonize new environments through sexual reproduction by the establishment of seedlings (Kittelson and Boyd, 1997; Nieva, 1996). The shape of the inflorescence, spikes and spikelets, and the amount of seeds that *S. densiflora* plants produce within a given geographic region are highly variable and have led to serious taxonomic confusion (Parodi, 1919; Mobberley, 1956; Bortolus, 2001). Site-specific genetic differences among and within populations may explain the morphological variation between regions (see Silander, 1979). However, the plasticity observed within populations of *S. densiflora* at Mar Chiquita coastal lagoon (Buenos Aires province, Argentina; Bortolus, pers. obs.) suggests that some environmental and ecological variables may regulate the plasticity of this species at a local scale (Bortolus, 2001).

The invertebrate community of *Spartina* marshes of the SW Atlantic is commonly dominated by the burrowing crab *Chasmagnathus granulata* Dana (1851, Grapsidae; Iribarne, 2001). This crab affects most of the edaphic conditions (including infaunal abundance and richness) commonly related to plant production, by reworking the sediments (Iribarne et al., 1997; Bortolus and Iribarne, 1999; Menone et al., 2000). They also selectively eat the new shoots of *Spartina densiflora*, causing a dramatic reduction of the standing crop in marshlands after disturbances such as cattle grazing or fire, and affect the general architecture of old marsh plants (Bortolus and Iribarne, 1999). *Chasmagnathus* can eat both old-plant litter and new shoots, and is able to shift to a strictly detrital diet whenever new shoots are not available, allowing it to prosper independently of how old the marshes are, or how frequently disturbed they are (Iribarne et al., 1997; Bortolus and Iribarne, 1999; Bortolus, 2001). Despite the large impact that *Chasmagnathus* has on plant biomass and on the entire habitat, it is unknown if it affects the sexual reproduction of *Spartina*.

In this paper we examine the hypotheses that *Chasmagnathus* affect the sexual reproductive effort of *Spartina densiflora*, and that the magnitude of this effect interacts with disturbance. To address these issues, we experimentally manipulated crab density and disturbance in one of the largest pristine marshes of Argentina for two years, and sampled crab–plant relationships across several sites with different intensities of crab activity and disturbance.

2. Materials and methods

2.1. Study area

The study was performed in Mar Chiquita (Argentina; Fig. 1), one of the most important coastal lagoons of southern South America, for its dimension, geomorphology, and faunal and floral diversity (see Iribarne, 2001). It is a body of brackish water affected by low amplitude tides (≤ 1 m; Spivak et al., 1994) and characterized by mudflats and large fringing salt marshes (Olivier et al., 1972). *Spartina densiflora* is the dominant species in the low and middle marsh, while in the high marsh, *Salicornia ambigua* is relatively more abundant (West, 1977). Disturbances such as fire and cattle grazing commonly denude small areas of aboveground *Spartina* biomass (Bortolus et al., 1999). Large groups of crabs populate areas commonly called “cangrejales” (from the Spanish for crab: “cangrejo”) and typically found throughout the intertidal zone in vegetated and unvegetated areas (with 60–100 ind m^{-2} ; Iribarne, 2001). The cangrejales are variable in extension and they may be affected by local disturbances such as the intense artisanal clam-farming or accidental fires. Consequently, some marsh areas can be devoid of crabs for several years before they are recolonized, even if plants are present and abundant.

2.2. Effect of crabs on the production of sexual reproductive structures of *Spartina*

To evaluate the effect of crabs on the sexual reproduction of *Spartina densiflora*, a caging experiment manipulating the density of crabs was conducted at La Boca Marsh (LBM; Fig. 1) from November 1997 to March 2000. Twenty-four permanent cages were deployed in the marsh. Each cage was 1 m \times 1 m and 0.6 m in height (plastic net; mesh size = 2 mm). The design of the cages was based on a one-year preliminary field test in a nearby marsh with different models of cages. Like previous studies with similar cages (e.g., Bertness, 1985; Bortolus and Iribarne, 1999), no cage-effect on plants was found after the one-year preliminary studies. Cages were sunken 10–20 cm into the sediment to prevent crabs from digging under them, and roots and rhizomes were cut with a knife around the perimeter of each cage to avoid

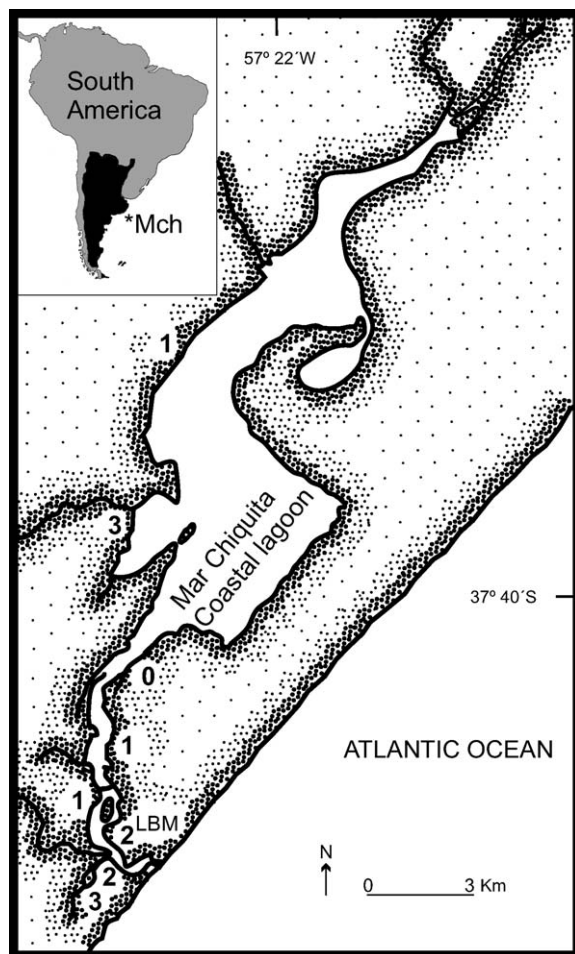


Fig. 1. Map showing the study sites within Mar Chiquita coastal lagoon. The numbers surrounding the lagoon indicate intensity of marsh disturbance (from no disturbance to highly disturbed): 0 = devoid of crabs and not affected by cattle or fires, 1 = burned and/or grazed and devoid of crabs, 2 = with crabs but never burned or grazed, and 3 = frequently burned and grazed and populated by crabs. Numbers also mark the sites used for seed collection. LBM indicates the location of La Boca Marsh, where the manipulative experiments with crabs were deployed. The map of South America in the upper left corner, shows the location of Mar Chiquita lagoon (Mch) on the coast of Argentina (in black).

resource reallocation through clonal integration from the outside. Cages were used in the following treatments. (1) Inclusion: crabs were included in burrows artificially made (we added 15–20 burrows to the natural density, with one crab per burrow). These experimental densities were within natural values (i.e., 70–100 ind m^{-2} ; Iribarne, 2001) but higher than in the control plots (see below). The cages were periodically checked to ensure that every burrow was active throughout the study (see Bortolus and Iribarne, 1999). (2) Exclusion: crabs were removed from their burrows by hand and standard crab-traps. These cages were periodically maintained to ensure that burrows were not active. (3) Control: cages with natural densities of crabs (~ 60 ind m^2). Control cages had open

spaces on the bottom to allow crabs to leave and/or enter. This method was tested during a preliminary test and found to be accurate. Treatments were replicated four times and fully interspersed (Hulbert, 1984). After two years we measured the length of the reproductive stems, the number of inflorescences and the number of seeds per inflorescence. We also measured seed viability ($n = 40$ per treatment). Seeds were pooled by treatment and the total proportion of viable seeds was estimated. The seeds were dried (70 °C until constant weight) and their weight was obtained by using a digital balance (± 0.0001 g) and compared among treatments. In order to evaluate the effect of crabs on the sexual reproduction of young *S. densiflora* plants, the same experimental design was repeated in the same salt marsh, but this time we clipped at ground level all the plants within every treatment plot. The experiments lasted from January 1st 1998 to March 31st 2000 and the clipping was made only once, at the beginning of the experiment (January 1st 1998). Treatments were replicated four times and fully interspersed (Hulbert, 1984). This method might not imitate the way that fire or herbivores can physically and chemically affect the plants (Hendrix, 1988), however, it allowed us to generate a contrasting scenario where we manipulated the plants and animals accurately and evaluated differences between treatments for the same variables.

2.3. Estimation of *Spartina* aboveground production

Since *Chasmagnathus* is known to eat *Spartina* (Bortolus and Iribarne, 1999), we evaluated the effect of *Chasmagnathus* on the production of aboveground structures and photosynthetic area of *Spartina densiflora* inside every plot of the caging experiment for two years. At the start of this study (January 1998), in the unclipped areas, plastic rings of the same color were placed in the base of every stem (within a 30 × 30 cm area) in order to identify them throughout the study period. Then, at each sampling period, each new stem was measured, quantified and labeled with a different colored plastic ring. Every dead stem was marked with another color to differentiate dead stems (with more than 3/4 of their surface brown-gray) from living stems. All labeled stems were individually measured at each sampling in height (± 0.001 cm) and basal diameter (± 0.0001 mm), and the number of leaves dead and alive was counted. The height and width of every leaf in those stems were also measured to quantify the live and standing dead matter. Measurements were made 40 days, and 12 and 24 months after the beginning of the experiment.

Aboveground biomass was indirectly estimated to avoid harvesting within the experimental plots. At each sampling period, the total dimension of each of 150 stems randomly chosen from the marsh was estimated as the product of basal width and stem length. They were then cut at ground level, measured to the nearest millimeter for

height, dried, and the mass was determined to the nearest milligram. From these samples, the relationship of mass and dimension was analyzed with and without a logarithmic transformation to find the mathematical regression equation that best fit the data (following Dai and Weigert, 1996). This equation was used to estimate the aerial biomass in the field experiment. A similar procedure was performed to estimate the dry weight of the leaves. The standing-live-stem biomass was estimated as the sum of the dry weight of all stems alive sampled inside of each cage, minus the weight of their dead leaves. The standing dead biomass was estimated as the sum of all standing dead stems and the dead leaves in the live stems.

Spartina densiflora stems are characteristically brown-yellowish, purple in the base and only the leaves appear to be able to photosynthesize. Thus, the photosynthetic surface within every treatment plot was estimated by quantifying the green-leaf surface of each stem measured. To estimate the total leaf surface of the plants, from 10 stems randomly selected inside of each cage all green leaves were measured in height and in width (measured in the center of the leaf). Then, the photosynthetic surface was estimated as the product of height and width, and converted to the stem density found in the experimental plot. To evaluate if there was a difference in the production of leaves among treatments after two years of the experiment, 10 stems of the first cohort of the clipped area were randomly collected and the number of leaves was quantified in April 2000. The leaves that senesced since the beginning of the experiment were quantified by counting the scars they left on the stem and statistically compared between treatments.

2.4. Relationship between crabs and the production of sexual reproduction structures of *Spartina*

To examine the relationship between the presence of crabs and the production of reproductive stems and inflorescences of *Spartina* in mature undisturbed stands, the following observations were performed at one of the largest pristine salt marshes in the lagoon (La Boca Marsh; Fig. 1) within two types of area, one naturally populated by crabs (i.e. density ~ 70 burrows m^{-2}) and the other devoid of them for several years (i.e. density < 5 burrows m^{-2}). The density of inflorescences of *Spartina* was estimated at random from 1×1 m quadrats ($n = 15$ per type of area). To evaluate phenotypic differences associated with seed dispersion, we measured the height of the reproductive stems within each one of the quadrats. The total dimension of every reproductive stem was estimated as the product of basal width and length. Randomly selected stem samples ($n = 15$ per type of area) were cut at ground level, measured to the nearest millimeter for height, dried ($70^\circ C$ until constant weight), and the weight of every reproductive stem was determined to the nearest milligram.

For *Spartina* in general, allocation is linked with physical and biological environmental variables that are known to be affected by *Chasmagnathus* (e.g., pH, sediment water content, soil community richness; Bortolus and Iribarne, 1999; Botto and Iribarne, 2000). A shift in the paths of resource allocation could either increase or decrease the amount of nutrients destined to seed development within the plant (e.g., Paige and Whitham, 1987). Thus, we investigated the hypothesis that crabs indirectly affect the development and/or viability of *Spartina* seeds by inducing changes in the resource allocation of the plant. First, to evaluate if there was any relationship between crab presence and seed viability, we estimated the proportion of viable seeds of *Spartina* within areas without (i.e., $0-6$ ind m^{-2}) and with crabs (six to seven marshes each; Fig. 1). One hundred and twenty seeds were collected at random from both types of area within the lagoon and tested for viability. Second, we evaluated if there was any relationship between the presence of crabs and seed weight (an indicator of seed development) by obtaining and comparing the dry weight ($70^\circ C$ until constant weight) of seeds from areas with and without crabs ($n = 60$ per area).

To examine the prediction that marshes with a combined effect of crabs and disturbance have the highest production of sexual reproductive structures, we estimated and compared the density of inflorescences of *Spartina densiflora* produced in eight salt marshes ($n = 10-15$ for each marsh, $1 m^2$ sampling area per plot) affected by different intensities of disturbance through their ontogenetic histories. The different disturbance intensities were (increasingly): (0) devoid of crabs and not affected by cattle or fires at least since December 1996 ($n = 1$), (1) burned and/or grazed (once, at the beginning of January 1996) and devoid of crabs at least since January 1996 ($n = 3$), (2) with crabs but never burned or grazed since January 1996 ($n = 2$), and (3) frequently burned and grazed (\geq once a year) and populated by crabs ($n = 2$; Fig. 1).

2.5. Data analysis

The null hypothesis of no effect on variables among treatments was evaluated with uni- and multivariate ANOVAs. A Tukey test (T) was performed to identify differences a posteriori, and the significance levels considered in all the analyses were not higher than 0.05 (Zar, 1999). The null hypotheses of no difference in variables between areas with and without crabs were evaluated with a Welch-approximation t -test (t_c ; Zar, 1999). Two-way repeated measures ANOVA were performed to analyze comparisons involving repeated measures at 40 days, 12 and 24 months. Repeated measurement analysis was not performed when assumptions were violated even after data were transformed (see Hulbert, 1984; Underwood, 1997).

3. Results

3.1. Effect of crabs on the production of sexual reproductive structures of *Spartina*

In the exclusion plots, all burrows rapidly collapsed because of lack of crab maintenance and tidal effect. The consequent lack of burrows within this treatment maximized the contrast with the other treatments. After two years, the reproductive stems were taller in the unclipped area ($F = 7.6$, $df = 1$, $p < 0.05$) and within the inclusion treatment plots ($F = 3.9$, $df = 2$, $p < 0.05$; Fig. 2A). No interaction was found ($F = 0.7$, $df = 2$, $p > 0.05$). The density of inflorescences was higher in the clipped plots than in the unclipped ones ($F = 10.9$, $df = 1$, $p < 0.001$), showing the highest values in the inclusion treatment ($F = 0.7$, $df = 2$, $p < 0.05$; Fig. 2B). Unclipped plots showed high variation in inflorescence density for the inclusion treatment, and non-significant differences among treatments. No interaction was found ($F = 0.2$, $df = 2$, $p > 0.05$). The number of seeds produced per meter square was higher in the clipped than in the unclipped area ($F = 11.8$, $df = 1$, $p < 0.05$), and the highest value was in the crab-inclusion treatment ($F = 4.5$, $df = 2$, $p < 0.05$; Fig. 2C). No interaction was found ($F = 1.8$, $df = 2$, $p > 0.05$). We found a higher proportion of viable seeds within the clipped area in comparison with those found in the unclipped area with the highest value in the inclusion treatment (Fig. 2D).

3.2. Effect of crabs on *Spartina* growth

Live, dead and total standing biomass were more abundant in both unclipped and clipped areas compared to the beginning of the experiment, but after two years clipped plots approached the values in unclipped areas (Table 1, Tukey test: $p \ll 0.001$; Fig. 3). The total photosynthetic area increased over time for both clipped and unclipped areas (Fig. 4). Only after 40 days the exclusion of crabs generated an increase in the photosynthetic area within both areas ($F = 11.2$, $df = 2$, $p < 0.001$; Fig. 4) but there were no differences among treatments within clipped and unclipped areas after one ($F = 2.5$, $df = 2$, $p > 0.05$) and two years ($F = 2.5$, $df = 2$, $p > 0.05$). The vegetative stems were higher in the unclipped areas; however, the stems in the clipped area increased their height over time and were approaching the height of stems in unclipped areas after two years (Fig. 4). There were no differences between clipped and unclipped areas after 40 days ($F = 3.3$, $df = 1$, $p > 0.05$), one year ($F = 0.96$, $df = 1$, $p > 0.05$) or two years ($F = 0.9$, $df = 1$, $p > 0.05$). No interaction was found ($F = 2.6$, 0.9 and 0.9 for 40 days, one and two years, respectively; always $df = 2$ and $p > 0.05$). The density of live stems was similar among treatments for clipped ($F = 2.7$, $df = 2$, $p > 0.05$) and unclipped

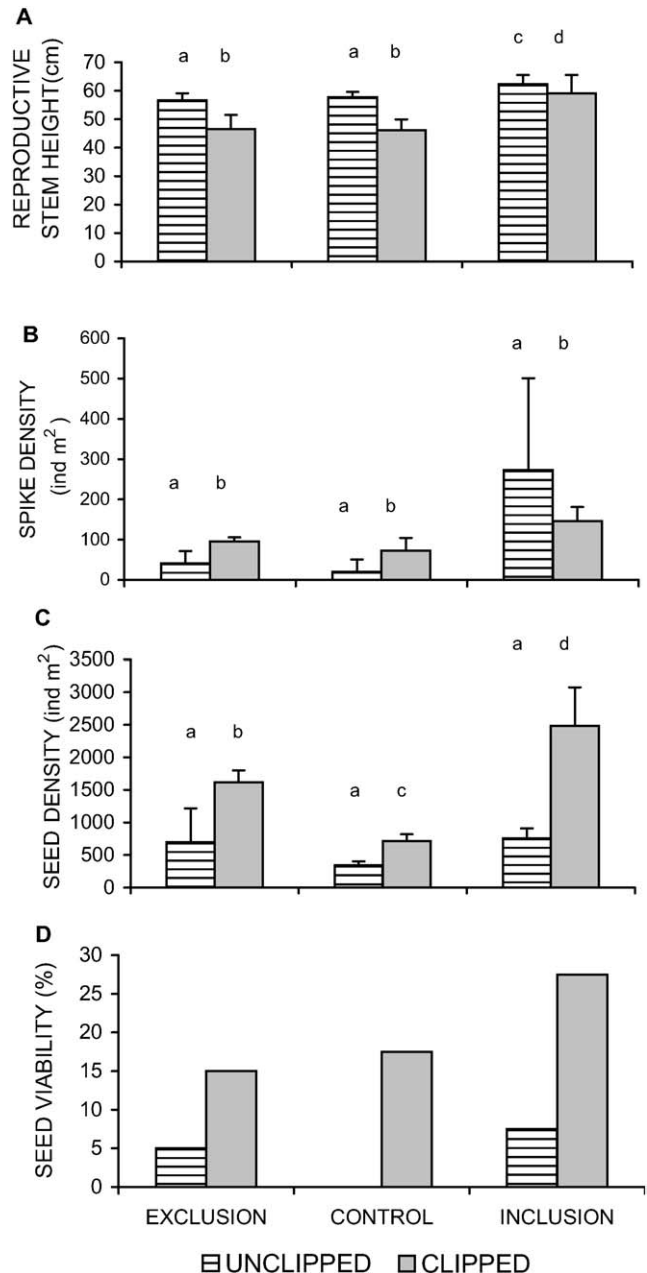


Fig. 2. Differences in the mean values of (A) reproductive stem height, (B) spike density, (C) seed density, and (D) seed viability after crab manipulation within clipped (gray bars) and unclipped (dashed bars) plots. Bars are $x + SE$. Different letters indicate statistical significance ($p < 0.05$).

($F = 0.6$, $df = 2$, $p > 0.05$) plants, but unclipped plots showed higher variation over time than clipped plots (Fig. 5A). Also the density of standing dead stems was higher in the unclipped area than in the clipped area the first year ($F = 11.9$, $df = 1$, $p < 0.01$; Fig. 5B). By that time, crab inclusion caused the highest density of standing dead stems for both clipped and unclipped plots ($F = 4.4$, $df = 2$, $p < 0.05$; Fig. 5B). However, only within the clipped plots the density of standing dead

Table 1

Two-way ANOVAs with repeated measures of *Spartina* biomass and vegetative stems at 40 days, one and two years in an experimental exclusion, control and inclusion of crabs within experimental clipped and unclipped marsh areas. C: clipping, Tr: treatments, T: time. ** $p < 0.01$, *** $p < 0.001$

Source		df	F
Standing live	C	1	9.73**
	Tr	2	0.20
	T	2	37.58***
	C × Tr	2	1.38
	C × T	2	8.26**
	Tr × T	4	0.29
	C × Tr × T	4	0.78
Standing dead	C	1	22.27***
	Tr	2	0.16
	T	2	61.36***
	C × Tr	2	0.44
	C × T	2	29.89***
	Tr × T	4	0.98
	C × Tr × T	4	0.33
Total standing	C	1	18.93***
	Tr	2	0.23
	T	2	71.9***
	C × Tr	2	1.15
	C × T	2	24.54***
	Tr × T	4	0.07
	C × Tr × T	4	0.77
Vegetative stem height	C	1	36.02***
	Tr	2	0.14
	T	2	31.54***
	C × Tr	2	0.03
	C × T	2	2.42
	Tr × T	4	0.38
	C × Tr × T	4	0.49

stems was significantly lower in the exclusion than in the other treatments (Tukey test: $p < 0.001$). A natural die-back was observed in the unclipped ($F = 38.1$, $df = 2$, $p < 0.001$) and in the clipped ($F = 19.7$, $df = 2$, $p < 0.001$) plots at the end of the second year (Fig. 5A) along with a massive removal of standing dead stems from both clipped and unclipped plots by the tide ($F = 43.3$, $df = 1$, $p < 0.001$; Fig. 5B). Two years after the clipping disturbance, the total number of leaves produced per stem was about 20% higher in the exclusion treatment ($x = 12$, $sd = 0.5$) than in the others (inclusion: $x = 9.5$, $sd = 2$, control: $x = 9$, $sd = 1.2$; $F = 6.045$, $df = 9$, $p = 0.02$, Tukey test: $p < 0.05$).

3.3. Relationships between crabs and *Spartina* sexual reproductive structures

The patterns of association we found in LBM between presence of crabs and reproductive structures of *Spartina* were consistent with our experimental results. Spikes were bigger in areas with ($x = 28.3$ cm, $sd = 3.1$) than in areas without crabs ($x = 16.9$ cm,

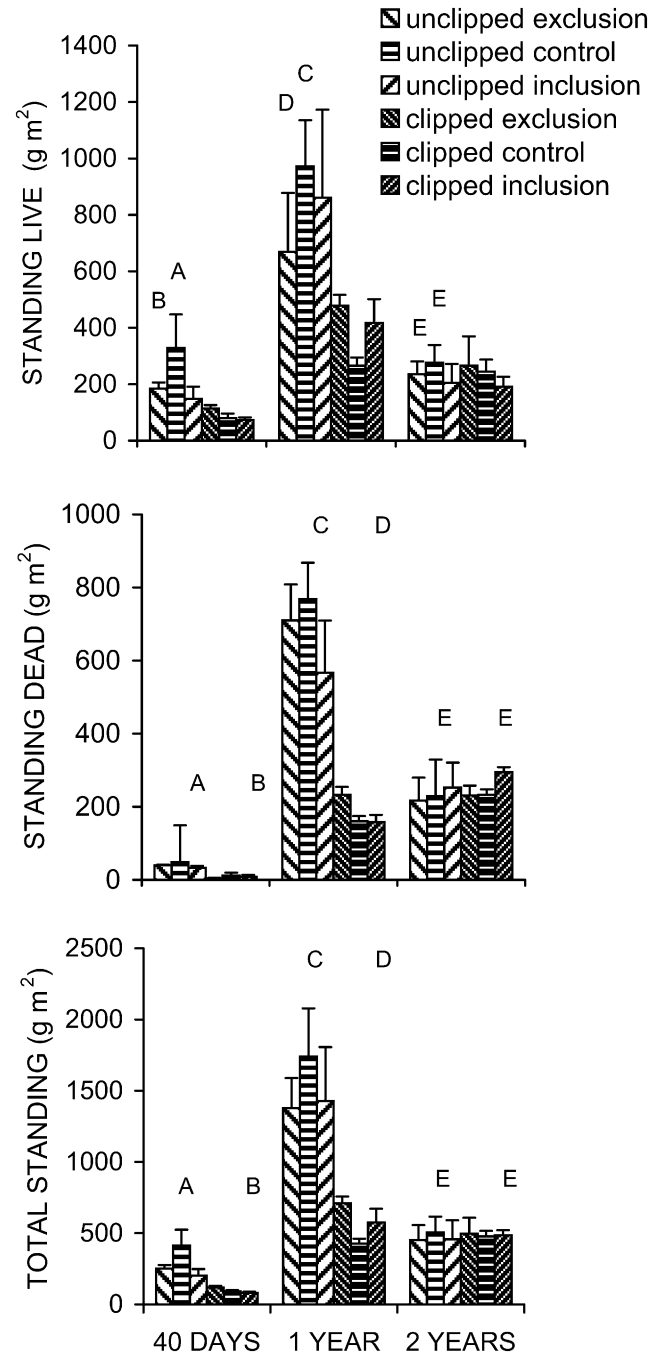


Fig. 3. Differences in standing live, dead and total biomass of *Spartina densiflora* after crab manipulation within clipped and unclipped plots during a two-year experiment. Bars are $x + SE$. Different letters indicate statistical significance ($p < 0.05$) between clipped and unclipped plots.

$sd = 4.3$; $t_c = 6.2$, $df = 14$, $p < 0.05$). This pattern was found for the weight of stems (with: $x = 4.6$ g, $sd = 1$, without: $x = 2.8$ g, $sd = 2$; $t_c = 6.2$, $df = 14$, $p < 0.05$) and height (with: $x = 67.5$ cm, $sd = 10.3$, without: $x = 48.4$ cm, $sd = 6.1$; $t_c = 6.64$, $df = 14$, $p < 0.05$). Inflorescence density had its maximal value in areas

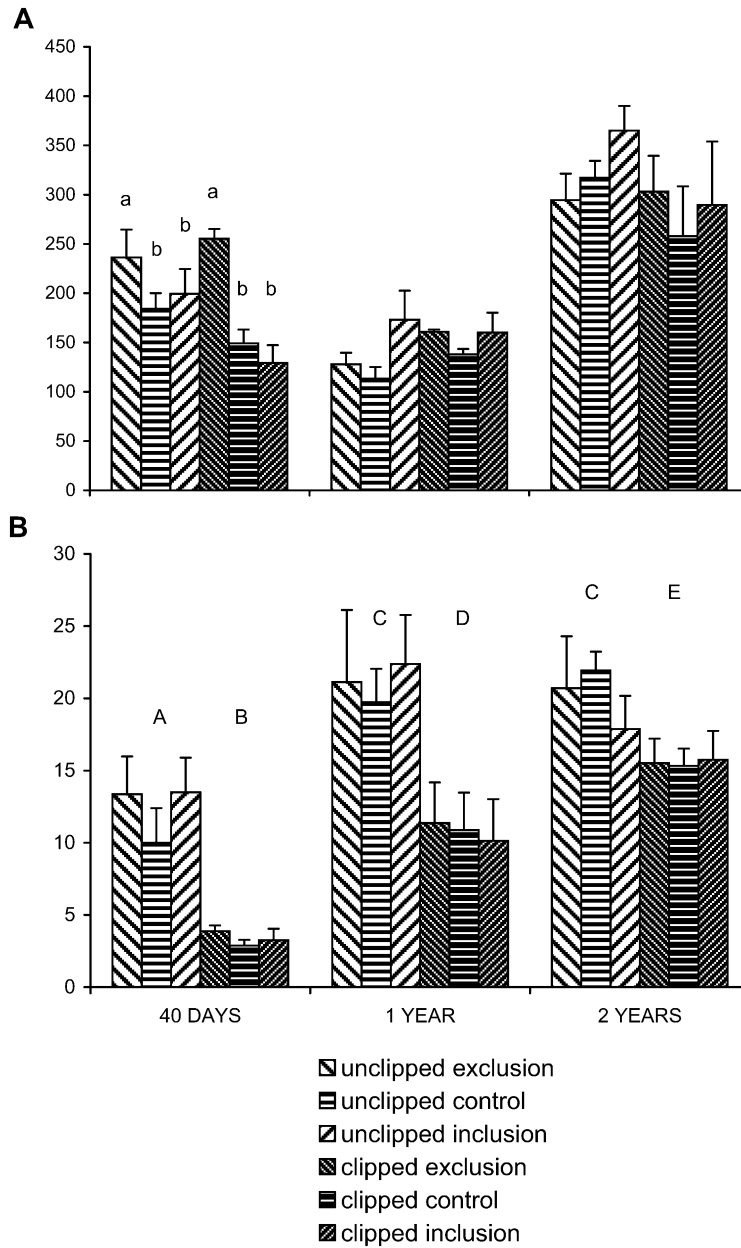


Fig. 4. Differences in total photosynthetic area (A) and non-reproductive stem height (B) of *Spartina densiflora* after crab manipulation within clipped and unclipped areas during a two-year experiment. Bars are $x + SE$. Different letters indicate statistical significance ($p < 0.05$) between clipped and unclipped plots (capital letters) and treatments (small letters).

with crabs ($x = 16.5 \text{ ind m}^{-2}$, $sd = 10.3$) but was not statistically different from that in areas without crabs ($x = 9.8 \text{ ind m}^{-2}$, $sd = 8.4$, $t_c = 1.58$, $df = 9$, $p > 0.05$). Thirty-three percent of the seeds were viable in areas populated by crabs while only 8% of them were viable in areas without crabs. Seeds did not differ in their weight between areas with ($x = 2.3 \text{ mg}$, $sd = 0.4$) and without crabs ($x = 1.8 \text{ mg}$, $sd = 0.6$; $t_c = 1.93$, $df = 18$, $p > 0.05$). The regional survey we performed was also consistent with our experimental results and sampling results at La Boca Marsh, corroborating the prediction on the

positive relationship between disturbance level and inflorescence production at a large scale too (Fig. 6).

4. Discussion

The agreement between our local and regional results strongly suggest that the phenotypic expression of sexual reproductive structures of *Spartina densiflora* at Mar Chiquita (Argentina) is mediated by the burrowing crab *Chasmagnathus granulata*. Even though genetic

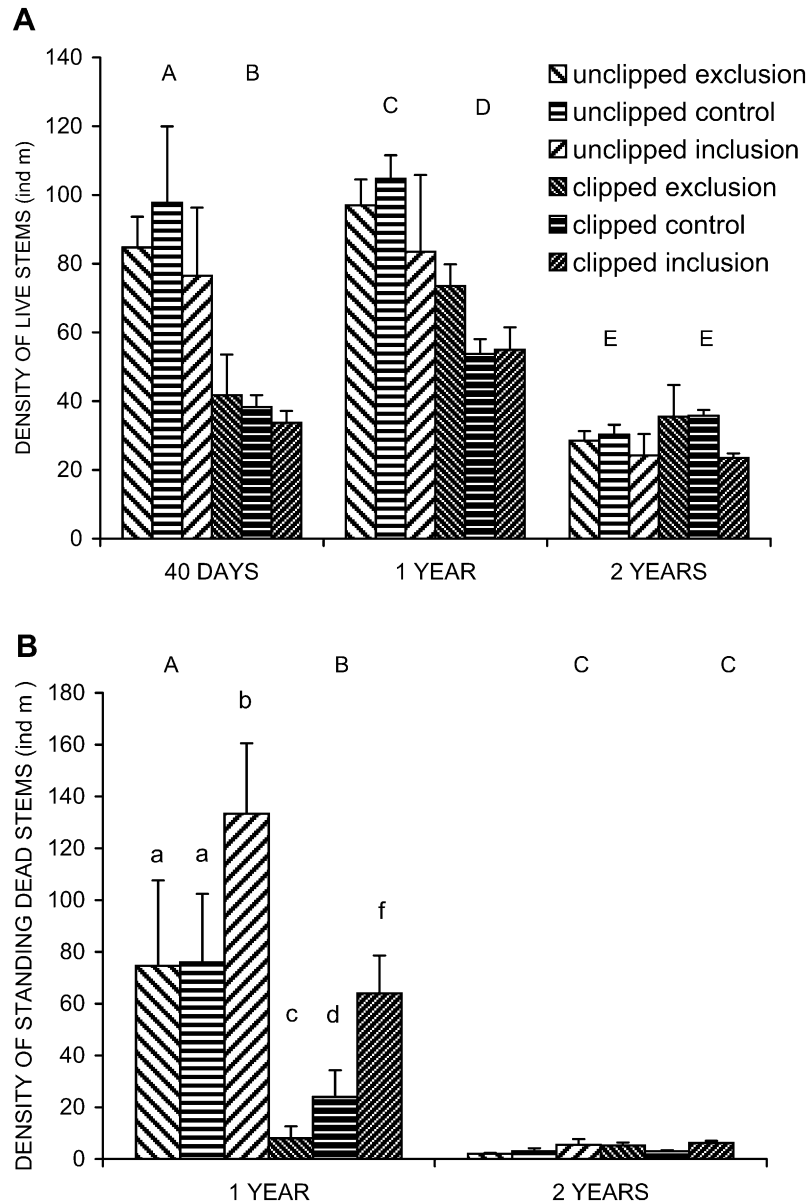


Fig. 5. Differences in density of live (A) and standing dead (B) stems of *Spartina densiflora* after the crab manipulation within clipped and unclipped areas during a two-year experiment. Bars are $x + SE$. Different letters indicate statistical significance ($p < 0.05$) between clipped and unclipped plots (capital letters) and treatments (small letters).

variability may explain some phenotypic differences among and within populations of *Spartina* (see Silander, 1979), our manipulative experiments show that crabs are able to generate significant differences at a local scale. The combination of crabs and the experimental clipping disturbance increased seed production by up to 721% when compared with areas without disturbance (i.e., unclipped control plots; see Fig. 2). Crabs also decreased the green photosynthetic leaf surface of clipped and unclipped *S. densiflora*, suggesting an important impact on plant production. Our experimental results matched the regional survey, corroborating the prediction that plants in undisturbed marshes have a lower sexual

reproductive effort than plants in highly disturbed marshes (Fig. 6). Most changes induced by crabs and environmental disturbances that we report in this paper affected characters with diagnostic value for *S. densiflora* (e.g., number, size and shape of seeds and leaf morphology). Therefore, it is likely that this site-specific variation may have led to the taxonomic confusion that caused the creation of the several taxonomic synonyms for this species (Parodi, 1919; Mobblerley, 1956; Bortolus, 2001). A major consequence of this confusion was the historic overestimation of the botanic biodiversity of South American salt marshes (see Mobblerley, 1956; West, 1977), and the consequent misunderstanding of the

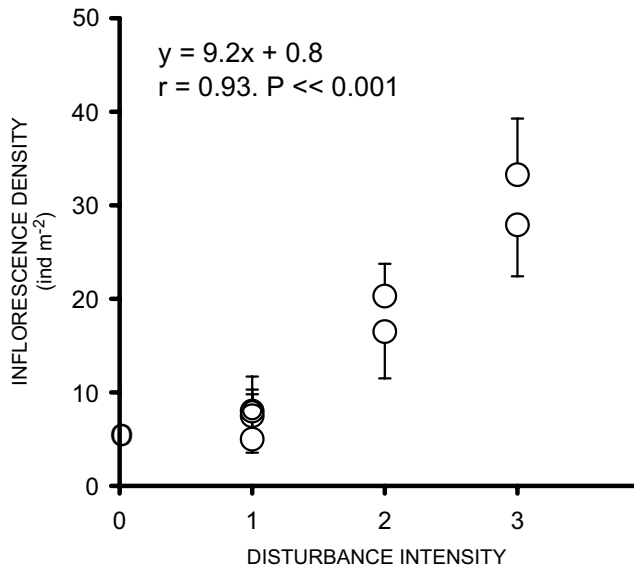


Fig. 6. Relationship between *Spartina*-inflorescence density and the intensity of marsh disturbance within Mar Chiquita coastal lagoon (Argentina). Each point corresponds to 10–15 measurements $\bar{x} \pm \text{SE}$ within a single marsh from a total of eight marshes.

ecologic patterns and processes that structure this environment (Bortolus, 2001).

4.1. *Spartina* phenotype and the crab effect

Plasticity allows morphogenetic variation in response to ecological and environmental factors and is particularly important in plants (Queitsch et al., 2002). For example, after being grazed, plants can allocate nutrients to recover the lost photosynthetic tissues (e.g., green leaves) or to the production of spikes and seeds (Harper et al., 1970; Howe and Westley, 1988). However, this resource allocation cannot be sustained forever and plants tend to die when grazed for long periods (Crawley, 1997). Our results showed that clipped plants generated more seeds than the unclipped ones independent of the crab effect. However, when we combined crab-inclusion and clipping disturbance, the clipped plants generated a greater number of seeds than plants in any other treatment. This variable sexual reproduction that we found in *Spartina densiflora* seems plausibly advantageous, maximizing the chance for a long-distance dispersion and settlement under particularly stressful ecological and environmental conditions. Although seed predation was not considered in our research, we did not observe potential seed-predators during this study. However, since the presence and abundance of seed-predators may vary strongly over time, further investigation should address this problem in more detail.

An increased production of seeds when a plant is severely stressed is usually implicitly interpreted as

a strategy that favors the persistence of the species in a given habitat after a disturbance event occurs (e.g., Harper, 1977; Schlichting, 1986). However, for *Spartina densiflora* this strategy might favor its dispersal rather than its persistence in the site. It is usually considered that the larger the number of seeds the higher the chance of persistence after a disturbance has occurred (Harper et al., 1970; Harper, 1977). Our results suggest that crabs might favor the dispersion and successful settlement of seeds of *S. densiflora* because they induce a large increase in the size of reproductive stems, in the seed production, and in the proportion of viable seeds. *Spartina* seeds combine a large flotation capacity (Saint-Yves, 1932; Bortolus, 2001) with a low germination rate in seawater (Nieva, 1996; Kittelson and Boyd, 1997), maximizing their transport through long distances in seawater and leading to a successful settlement (e.g., UK–France: Gray et al., 1990). Therefore, by mediating the expression of *Spartina* phenotypes that increase its sexual reproduction and long-distance dispersion, *Chasmagnathus* could indirectly be inducing changes in the community composition and general structure of distant marshes (and/or other coastal environments) without even being present.

After a disturbance such as fire occurs, *Chasmagnathus* crabs eat *Spartina*, reducing its aboveground biomass by up to 87.5% (Bortolus and Iribarne, 1999). They also increase the standing dead biomass of *Spartina* (this paper). Our results show that despite the effect of crabs on the leaf morphology, standing dead biomass and live biomass, the clipped plants gradually approximated that of unclipped plants. This suggests that in Mar Chiquita coastal lagoon, *Spartina densiflora* may eventually escape from the effect of crabs by continuing to grow during the winter, when crabs show the lowest metabolic activity (Bortolus, 2001; Iribarne, 2001). Considering that crabs prefer young soft tissues over old tissues (Bortolus and Iribarne, 1999; this paper), the impact of crabs on *S. densiflora* seems to be modulated by the timing and frequency of occurrence of those disturbances that control the maturity of the plants (e.g., fires and clipping). This crab-impact seems to be maximal in the short-term right after a disturbance occurs, during the crab-active period of the year.

4.2. Crab-mediated phenotypic changes in *Spartina* and its relevance in marsh community ecology and environmental biology

Spartina plants are known to regulate the distribution of their neighbors through competition (Pennings and Bertness, 2001) and/or facilitation (Bertness and Callaway, 1994). The complexity of the spatial structure of these plants and their variable nutritive quality are also known to modulate the abundance and distribution of several aerial, terrestrial (above- and belowground),

aquatic and marine organisms (Valiela, 1995; Day et al., 1989; Bortolus et al., 2002). Therefore any major factor able to affect *Spartina* structure and/or production would indirectly affect the whole community (Silliman and Bortolus, 2003). Burrowing invertebrates, for example, can alter dramatically the edaphic characteristics of the substrate related with *Spartina* production (Bertness, 1985; Takeda and Kurihara, 1987; Bortolus and Iribarne, 1999; Talley et al., 2001), change the composition of the associated soil community, directly decrease the primary production, and alter plant's phenotype and physiology in the marshes where they live (Bertness, 1985; Iribarne et al., 1997; Bortolus and Iribarne, 1999; this paper). In particular, *Chasmagnathus* crabs are among the few organisms known able to affect most of these variables at once (Bortolus and Iribarne, 1999; Bortolus, 2001). However, more research is needed to understand the effects generated by the burrowing activity and the crab herbivory separately. The separation of these two processes might be especially useful in the study of the environments invaded by *Spartina densiflora* where burrowing-herbivore animals may not be present. In New England (USA) salt marshes, the removal of the fiddler crab *Uca pugnax* generated a decrease of about 50% in the number of flowering stems of *Spartina alterniflora* (Bertness, 1985). However, *U. pugnax* is not a herbivore (Crane, 1975), suggesting that burrowing activity alone may induce the increase of *Spartina* flowering and seed production. This is an important inference considering that burrowing organisms may be more common than *Spartina*-eaters in salt marshes worldwide. A higher seed production might favor *Spartina* seed dispersal and the colonization of other marsh sites (Saint-Yves, 1932), and it is likely to explain the high success and fast spreading recently shown by *S. densiflora* within invaded areas worldwide (Spain: Nieva, 1996; USA: Smith et al., 2002). Because *Spartina*-animal associations are highly specific (Lana and Guiss, 1991; Netto and Lana, 1999), small changes in the plant composition of a marsh tend to affect the faunal composition too (e.g., Chung, 1993). Our results suggest that after *S. densiflora* has been introduced the effects of native burrowing/herbivore invertebrates might have macro-ecological impact, for they are able to induce changes in the structure of other region's communities by amplifying *S. densiflora* invasiveness. This kind of complex long-distance interactions are commonly overlooked by biologists studying the processes regulating the community structure of coastal environments, since they traditionally focus only on species present in a particular community (or communities) during or before the study is conducted. Our results suggest that the environmental context and ecological interactions having place at a particular site may trigger the expansion process of a given species and generate the colonization of distant sites. Considering

that this process could involve the massive invasion of an entire region after a punctual isolated introduction, more attention should be directed to this kind of long-distance interaction in discussions of applied ecology and environmental management.

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