Marine Environmental Research 71 (2011) 122–130

Contents lists available at ScienceDirect

Marine Environmental Research

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

Relationship between anthropogenic sewage discharge, marsh structure and bird assemblages in an SW Atlantic saltmarsh

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ARTICLE INFO

Article history:
Received 10 June 2010
Received in revised form
6 December 2010
Accepted 7 December 2010

Keywords:
Saltmarsh bird
Spartina alterniflora
Eutrophication
Coast
Bahía Blanca estuary
Argentina

ABSTRACT

One of the main effects of urbanization on coastal areas is through the discharge of sewage, which increases nutrient concentrations in the receiving environment. Salt marshes, like other coastal marine environments, are limited by nutrients, mainly nitrogen, and thus increasing nutrient loadings to a marsh may have consequences on marsh characteristics. We evaluated how the effects of nutrient enrichment in the form of sewage input, affected the vegetation structure and bird assemblages in a *Spartina alterniflora* salt marsh system near Bahía Blanca, Argentina (39° 01′ S – 56° 25′ W). Surveys of nutrient concentration, vegetation and birds were made at three different distances from the sewage discharge source. The concentration of ammonium, phosphate, and nitrate and the percent organic matter was higher in marshes nearest to the sewage discharge source. Bird composition and abundance, and vegetation physiognomy changed along a gradient of nutrient concentration. The increased habitat complexity found near the areas of higher nutrient concentration was exploited by birds that use neighboring interior and coastal habitats, including *Spartina densiflora* marshes, freshwater marshes and upland shrubby habitats. Our results show that local increases of nutrient inputs directly changed the vegetation physiognomy, and indirectly the composition and abundance of bird assemblages.

1. Introduction

The human population tends to live near the coastline (Wolanski, 2007), and thus, human activities can have greater impacts on the coastal ecosystems than on inland habitats (UNEP, 2006; Valiela, 2006). One of the main results of coastal populations is the discharge of sewage, which involves increased nutrient concentrations in the coastal environment (GESAMP, 1990). Salt marshes, like other coastal ecosystems, have relatively low-nutrient supplies (Valiela and Teal, 1974), and so nutrient increases can have large consequences i.e., changing the (1) structural complexity of marsh plants by enhancing biomass and stem density, (2) species composition, and (3) zonation patterns (Levine et al., 1998; Daleo et al., 2008). Also, nutrient increases may lead to marsh subsidence and habitat fragmentation, depending on the balance between sediment depositions, increased plant production, and increased decomposition (Valiela and Teal, 1974; Harper, 1995; Deegan, 2002). Specifically, in salt marshes characterized by large monospecific plant stands, nutrient enrichment may modify the plant physical structure by changing the plant growth form (Deegan, 2002). Nitrogen is usually the limiting nutrient controlling aboveground salt marsh vegetation production (Mendelssohn, 1979) and its availability increases the plant canopy and standing stocks, which is followed by higher rates of vegetation decay (Mitsch and Gosselink, 2000), and changes in species composition (Craft et al., 1995; Vaithiyanathan and Richardson, 1997). A case largely studied in the northern hemisphere is the change of the dwarf form of *Spartina alterniflora* into the tall form as a result of an increase in nutrients loading to a marsh (Valiela and Teal, 1974; Valiela et al., 1978). This increased nitrogen loading, however may or may not lead to a net gain of the marsh depending on the balance between the change in marsh plant production and decomposition. Increases in the primary production or vegetation biomass may produce more detritus (Harper, 1995; Deegan, 2002) which is important in food webs (Deegan et al., 2000) and in creating peat that forms the physical structure of the marsh platform (Friedrichs and Perry, 2001). Dead vegetation deposition ('wracks') on coastal marshes is a natural disturbance that can increase habitat heterogeneity (Valiela and Rietsma, 1995; Minchinton, 2002). Thus when nutrients loadings to a salt marshes increase we expect greater vegetation heterogeneity because of the increased canopy structure and more frequent occurrence of wracks.

Vegetation structure, composition, and floristic characteristics are substantial factors influencing bird habitat selection, because

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doi:10.1016/j.marenvres.2010.12.003

these factors provide food, nesting sites, and cover from predators (Macnally, 1990; Vickery et al., 2001; Buchanan et al., 2006). Individuals select habitats that are an optimal combination of resources that allows them to perform multiple activities (e.g., foraging, breeding, roosting; Hilden, 1965; Fretwell and Lucas, 1970; Block and Brennan, 1993; Steele, 1993). Thus habitat structural heterogeneity is often a good predictor of bird diversity (Mac Arthur and Mac Arthur, 1961; Wiens, 1973; Roth, 1976). This result is probably due to the role of habitat complexity in promoting niche diversification (Wilson, 1974; Roth, 1976). There are many examples of the effect of nutrient enrichment on the aquatic environment (i.e., lakes estuaries; Robledano et al., 2008), such as those generated by algal blooms on waterbirds (Rosa et al., 2003), waterbird breeding (Rönkä et al., 2005) and shorebirds (Raffaelli, 1999; Lopes et al., 2006). Bird abundance is positively correlated with lake nutrient levels (Nilsson and Nilsson, 1978; Hoyer and Canfield, 1990, 1994; McCarty, 1997). However, although the effects of eutrophication are a well studied phenomena worldwide (Vitousek et al., 1997; Valiela, 2006), we are not aware of studies on the effects of nutrient enrichment on bird assemblages associated with salt marsh habitats.

The salt marshes of the SW Atlantic are being modified at an increasing rate (Costa et al., 2009), primarily by cattle grazing, fire (Isacch et al., 2004), and sewage discharges (Nebbia and Zalba, 2007; Martinetto et al., 2010). As happens elsewhere, in this region there are sewage discharges into the salt marshes. Specifically, there is an important sewage discharge on a *Spartina alterniflora* salt marsh in Bahía Blanca (Argentina) where salt marshes develop characteristics of those of the SW Atlantic (Isacch et al., 2006). Because increased nutrients on a salt marsh are expected to increase vegetation structure from the direct consequences of eutrophication (Daleo et al., 2008), we predicted that bird assemblage will also change from the indirect influences. Thus, we studied how nutrients change along a gradient from the sewage discharge source, and how this gradient is related with the vegetation structure and bird composition and abundance.

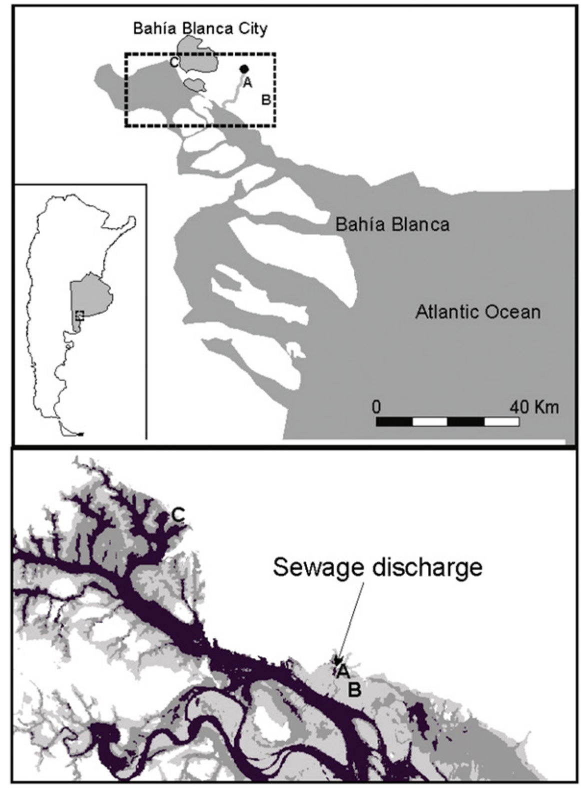

Fig. 1. Top: Bahía Blanca (Argentina) during a low tide. White doted lines enclose the amplified area included in the bottom image. The black dot represents the location of the sewage discharge source. The inset in the lower left shows the location of the study area in Argentina. Bottom: a detail of the sewage discharge source and the three sites sampled (A, B and C) in *Spartina alterniflora* salt marshes from Bahía Blanca estuary. The four main habitats identified here are uplands (white), salt marshes (light grey), mudflat (dark grey) and sea (black). Letters indicate the location of the three sites studied: (A) the Naposta marsh located near the discharge site; (B) the Midway marsh, located 200 m from the discharge source; and (C) the Maldonado marsh 10 Km from the discharge source.

2. Materials and methods

2.1. Study area

The study was performed at the Bahía Blanca estuary (39°01′S-56°25′W; Argentina), which includes one of the largest SW Atlantic salt marshes (Isacch et al., 2006; Fig. 1). The bay has a total area of 2300 km^2 (Montesarchio and Lizasoain, 1981), with 10% of that surface covered by salt marshes and 20% by mudflats (Isacch et al., 2006). The salt marsh vegetation is dominated by two species; *Spartina alterniflora* grows in the lower marsh, and *Sarcocornia perennis* grows in the upper marsh. The industrial and port city of Bahía Blanca (300,000 inhabitants) is located on this coast. This city discharges its sewage into the bay relatively far from the city (Fig. 1). As in most of the bay, a large salt marsh dominated by *S. alterniflora* develops around the discharge point. Our observations suggested that there are strong variations in the salt marsh vegetation characteristics linked with the sewage discharge, and that they are not typical of a natural gradient. The latter conclusion is supported by a study about biomass variation of *S. alterniflora* marshes developed in that region (Isacch et al., 2007), in which it was found that *S. alterniflora* plants reached larger sizes near the sewage discharge.

Three sites were selected at different distances from the sewage to study the relationship between anthropogenic nutrient concentration, the vegetation and birds, (Fig. 1). The salt marsh growing around the sewage discharge pipe and the proximal part of the discharge focus, was considered as the area of maximum nutrient effect (Naposta marsh, 10 ha; Fig. 1). A second site located approximately 200 m from that source was considered as an area of medium effect (Midway marsh, 11.5 ha; Fig. 1). A third site located at 10,000 m from the main discharge source was considered as an area of low effect (Maldonado marsh, 14.5 ha; Fig. 1). All marshes have similar tidal conditions, given that they are positioned to the SW, and were located at the same distance (~1500 m) from the main tidal channel. We assumed, therefore, that the three marshes are similar except for their exposure to the sewage outfall.

2.2. Nutrient levels

To evaluate if the sewage discharge caused nutrient enrichment of the soil, we collected sediment samples to determine the total organic matter content (OM), concentration of dissolved inorganic nitrogen (nitrate and ammonium), and concentration of inorganic phosphorous (i.e., phosphate) in porewater. Twenty sediment samples were obtained using a stratified random sampling design at different distances from the sewage channel: 20 m and 80 m (Naposta marsh), 150 and 220 m (Midway marsh), and 10,000 m (Maldonado marsh). Sediment samples were collected by pushing a PVC core (3 cm diameter, 8 cm length) into the sediment. Cores were immediately transported to the laboratory, where porewater was obtained by centrifugation (9000 × *g*, 10 min), filtered, and stored at −20 °C until the analysis of dissolved nutrients. The nitrate concentration in porewater was determinated calorimetrically as nitrite after reduction by cadmium followed by diazotization (Strickland and Parsons, 1968). The concentration of ammonium was measured using the blue indophenol method (Solórzano, 1969). The concentration of dissolved phosphate was measured using the molybdate method (Strickland and Parsons, 1968). A set of samples were dried (7 days at 60 °C) and combusted (8 h at 500 °C) to determine the total organic matter content (%) as the loss weight between dry and combusted sediment. The null hypothesis of no difference in nutrients (nitrate, ammonium and phosphate concentrations, and organic matter content) at different distances of the sewage discharge source was evaluated by using a one-way ANOVA. An *a posteriori* LSD Fischer test was used to identify differences when necessary (Zar, 1999). The relationship between nutrients concentrations and distance to the sewage discharge source was assessed by simple regression analysis (Zar, 1999).

2.3. Vegetation and bird surveys

We sampled vegetation and birds along 7 randomly distributed 100 × 60 m transects in each marsh using the fixed-width strip-transect method (following Bibby et al., 1997). Transects were separated by a minimum distance of 100 m. Birds were surveyed monthly between July 2007 and June 2008. The habitats included within transects were the vegetated marsh platform and the open gaps (determined by dead vegetation deposition, i.e. wracks). The vegetation physiognomy was surveyed in each bird sampling unit (i.e., transect) during winter and summer. In each transect used to survey birds, we measured the following vegetation variables: height of the dominant strata of vegetation, plant species, percent of total and green coverage vegetation, and the number of *Spartina alterniflora* spikes (i.e., inflorescences). The vegetation data for each transect came from the average of two squares (1 × 1 m) randomly located along transect. All vegetation surveys were made in vegetated areas. We assumed that the vegetation height and cover represented the main components of the habitat structure in marsh ecosystems (Cardoni et al., 2007).

The null hypothesis of no difference in bird richness and abundance and vegetation characteristics (height of vegetation, percent coverage of *Spartina alterniflora* and spike density) among the three sites at different distances of the sewage discharge source was evaluated by using a one-way ANOVA for each sampling date. An *a posteriori* LSD Fischer test was used to identify differences, when necessary (Zar, 1999). The null hypothesis of no difference in vegetation characteristics between seasons was evaluated by using a *t*-test (Zar, 1999). We used simple regression analysis (Zar, 1999) to assess the relationship between nutrient concentrations and vegetation variables, and between bird and vegetation variables.

There were dead vegetation depositions of *Spartina alterniflora* ('wracks') in areas close to the sewage channel. To evaluate if marshes with different nutrient levels have a different density of wracks, we compared the number of wracks among them using the Google Earth software (http://earth.google.com). The study site was covered by an image from Google Earth with high resolution (date 01-12-2007) where wracks previously geopositioned were easily recognized. Then a portion of 10 ha in the core area was analyzed for each salt marsh (Naposta, Midway and Maldonado). Wracks in each image were digitized to determine the number and the cover area. The density of wracks was then calculated in each case from the digitized images and differences among sites were evaluated using a Chi-square test (Zar, 1999).

3. Results

3.1. Nutrient levels

There was a nutrient gradient linked to the distance from the sewage discharge source. Ammonium, phosphate and nitrate concentrations were higher at Naposta, the marsh nearer to the sewage discharge, and lower in Midway and Maldonado marshes (ANOVA; Ammonium: $F_{2,16} = 16.90$, $P < 0.05$; Phosphate: $F_{2,16} = 14.01$, $P < 0.05$; Nitrate: $F_{2,16} = 47.91$, $P < 0.05$; Fig. 2). The same pattern was recorded for the OM content (ANOVA; OM: $F_{2,16} = 4.73$, $P < 0.05$; Fig. 3).

Nutrient concentrations decreased with increasing distances from the sewage discharge source (Ammonium: $r^2 = 0.34$, $P < 0.05$; Phosphate: $r^2 = 0.30$, $P < 0.05$; Nitrate: $r^2 = 0.47$, $P < 0.05$) and no relationship was found between distance and OM ($r^2 = 0.17$, $P > 0.05$).

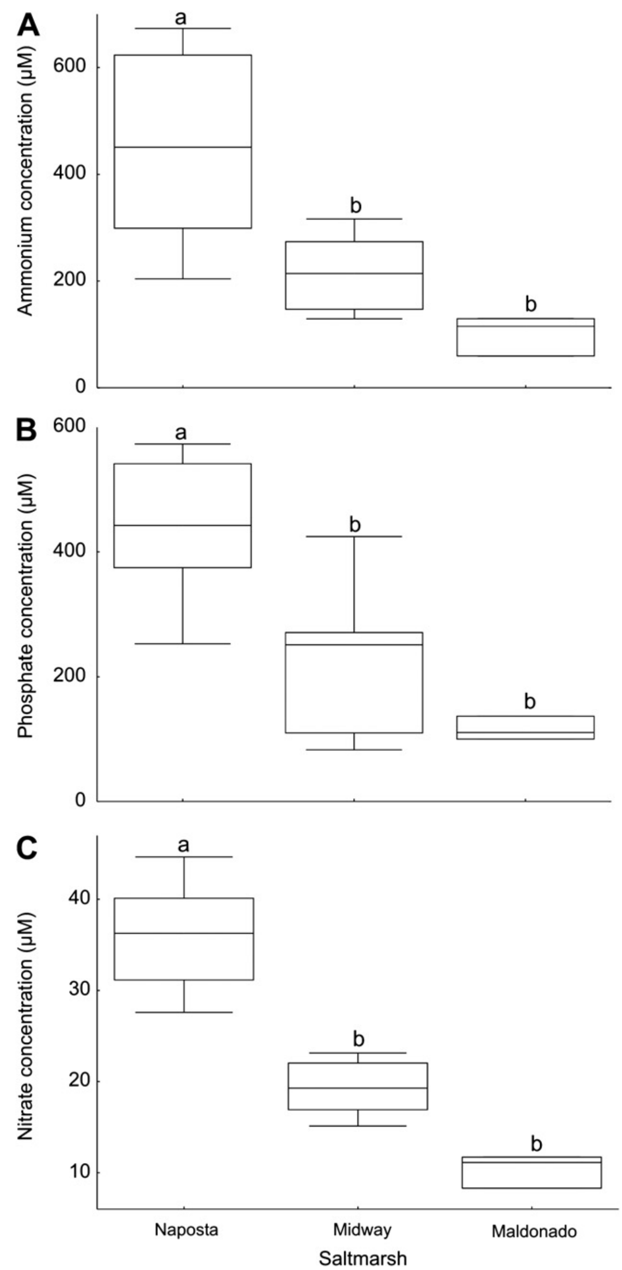

Fig. 2. The concentration of ammonium (A), phosphate (B) and nitrate (C) in sediment in three salt marshes at different distances from the sewage discharge source (Naposta, Midway, Maldonado; see Fig. 1). The box plots are constructed with the limits of boxes being the 75th and 25th percentiles, and lines represent the minimum and maximum value; the line inside the boxes is the median value. Different letters in the upper the plot represent significant differences as determined using an *a posteriori* LSD test ($P < 0.05$).

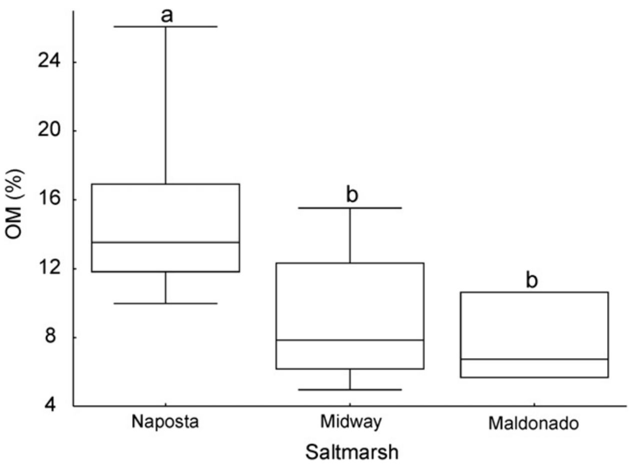

Fig. 3. Percentages of organic matter (OM) in sediments in three salt marshes at different distances to the sewage discharge source (Naposta, Midway, Maldonado; see Fig. 1).

3.2. Vegetation

The only species recorded in all studied sites was the cord grass *Spartina alterniflora*. The vegetation height and cover were higher in Naposta marsh than in the Midway and Maldonado marshes, both in winter and summer (ANOVA; Vegetation height; winter: $F_{2,18} = 62.85$, $P < 0.05$; summer: $F_{2,18} = 18.40$, $P < 0.05$; Vegetation cover; winter: $F_{2,18} = 23.79$, $P < 0.05$; summer: $F_{2,18} = 10.41$, $P < 0.05$; Fig. 4A and B). The vegetation height was higher at the Midway marsh than at the Maldonado marsh only during winter. Plant cover was higher at the Midway marsh than at the Maldonado marsh in the summer. The spike density was higher both in summer and winter in the Naposta and Midway marshes than in the Maldonado marsh (ANOVA; winter: $F_{2,18} = 9.92$, $P < 0.05$; summer: $F_{2,18} = 8.95$, $P < 0.05$; Fig. 4C).

The total and green cover of *S. alterniflora* were both higher in summer than winter at the three salt marshes (*t*-test; total cover, Naposta, $t_{12} = 2.95$, $P < 0.05$; Midway, $t_{12} = 2.67$, $P < 0.05$; Maldonado: $t_{12} = 5.18$, $P < 0.05$; green cover, Naposta, $t_{12} = 10.81$, $P < 0.05$; Midway, $t_{12} = 4.31$, $P < 0.001$; Maldonado, $t_{12} = 8.01$, $P < 0.05$). Vegetation height was better explained by nutrients concentrations (Phosphate, $r^2 = 0.66$, $P < 0.05$; Ammonium, $r^2 = 0.70$, $P < 0.05$; Nitrate, $r^2 = 0.84$, $P < 0.05$, Fig. 5A) than vegetation cover (Phosphate, $r^2 = 0.51$, $P < 0.05$; Ammonium, $r^2 = 0.51$, $P < 0.05$; Nitrate, $r^2 = 0.66$, $P < 0.05$).

There were 13 wracks (7.5% of 10 ha marsh) at the Naposta marsh, 3 wracks (1.9% of 10 ha marsh) at the Midway marsh, and none at the Maldonado marsh (Chi-Square = 227.63, df = 1, $P < 0.05$).

3.3. Birds

Nineteen bird species were recorded in the three sites (Table 1). The total bird species recorded for each distance were 16, 12 and 5 species in the Naposta, Midway and Maldonado marshes, respectively. Seventy-two percent of all birds, at all sites was at the Naposta marsh (the nearest area of the discharge source) and only 23 and 5% at the Midway and at Maldonado marshes, respectively. The bird abundance was always higher in Naposta marsh than in Maldonado marsh (Table 2 and Fig. 6). The bird abundance exclusive of the Yellow-winged Blackbird also was higher in the Naposta marsh than in the Maldonado marsh for all months (Table 2 and Fig. 6). In some months it was also higher in the Naposta marsh than in the Midway marsh (Table 2 and Fig. 6). The bird richness was always higher in the Naposta marsh (Fig. 6).

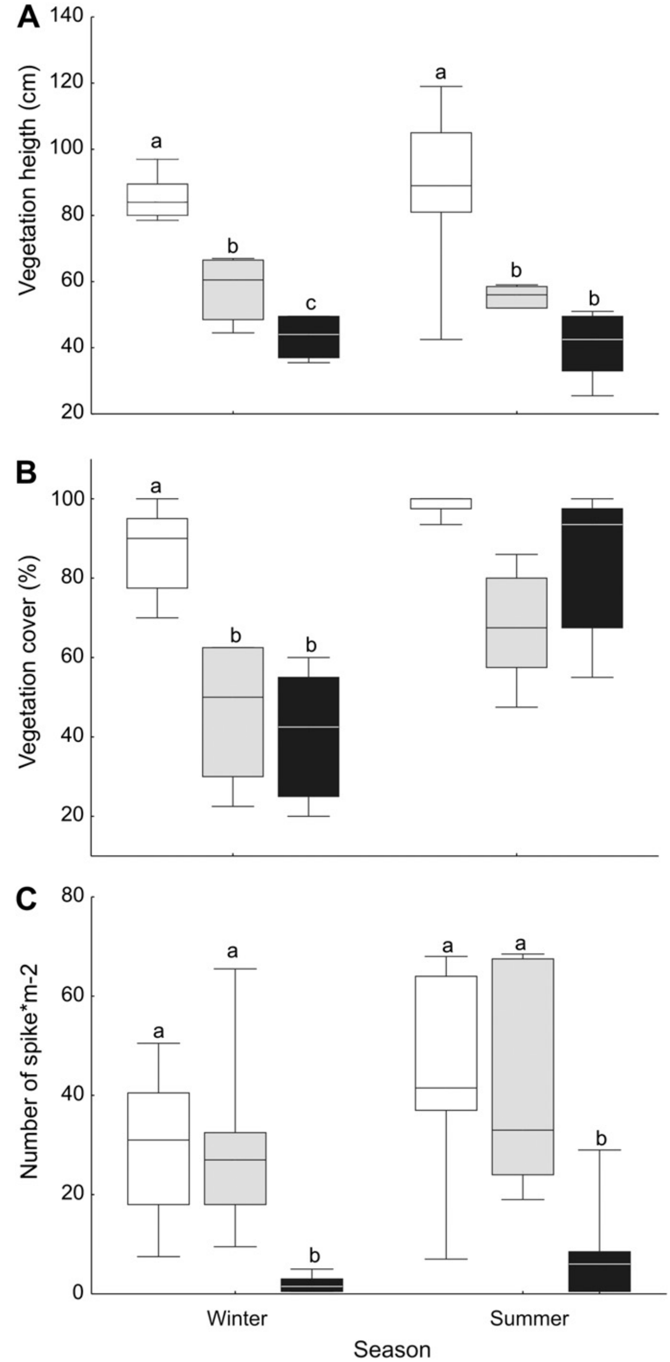

Fig. 4. Height (A), cover (B) and spike density (C) of *Spartina alterniflora* in three salt marshes at different distances to the sewage discharge source (Naposta, open box; Midway, grey box; Maldonado, black box; see Fig. 1).

The Yellow-winged Blackbird (*Agelaius thilius*) was the most abundant bird species, with 72% of the total bird abundance. However, 80% of the Yellow-winged Blackbird abundance was recorded only in the Naposta marsh. Other bird species associated with tall grass (without considering Yellow-winged Blackbird; Table 1) were only recorded at the Naposta marsh, the site with higher values of *S. alterniflora* cover and height. Bird species associated with open areas (i.e., short grass and mud flats; Table 1) had a higher abundance in the Naposta marsh than in the Midway and Maldonado marshes in four of ten months, and were higher in two months in the Naposta and Midway marshes than in Maldonado

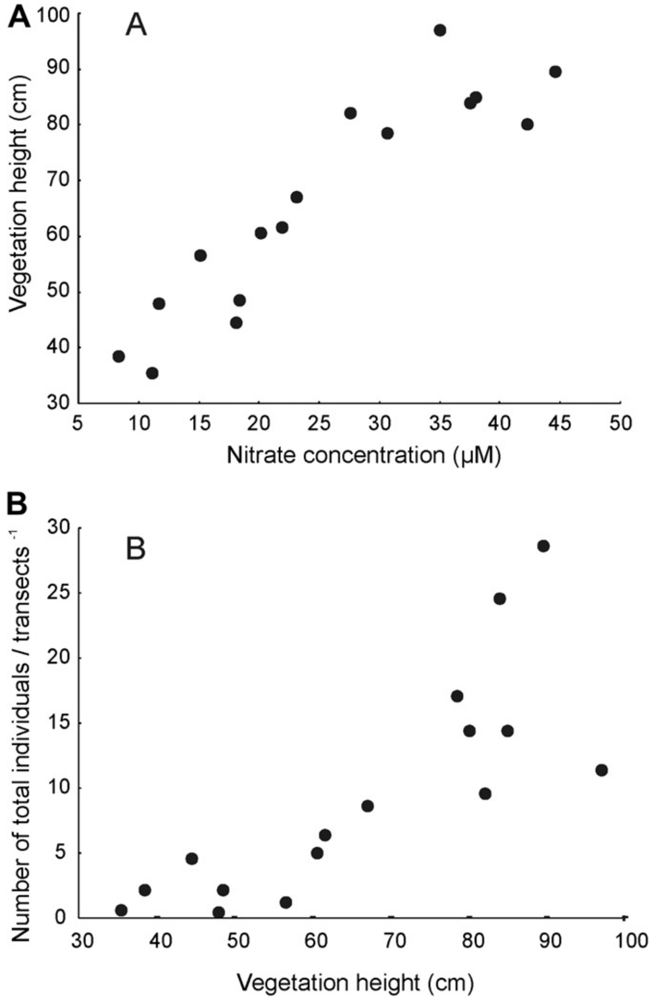

Fig. 5. Relationship between vegetation height and nitrate concentration (A), and vegetation height and total number of individuals of birds (B).

marsh (Table 2). It should be noted that there were more wracks in the Naposta marsh than in the other two marshes.

Bird abundance and richness were both positively correlated with the height of *Spartina alterniflora* (Abundance**S. alterniflora* height: $r^2 = 0.65$, $P < 0.05$, Fig. 5B; Richness**S. alterniflora* height: $r^2 = 0.28$, $P < 0.05$). Bird abundance was also explained by *S. alterniflora* cover ($r^2 = 0.53$, $P < 0.05$). Bird richness was not correlated with vegetation cover ($r^2 = 0.17$, $P > 0.05$). Bird abundance without Yellow-winged Blackbird (species that represented the 72% of the total bird abundance) was correlated with vegetation cover and height (Abundance without Yellow-winged Blackbird* *S. alterniflora* height: $r^2 = 0.47$, $P < 0.05$; Abundance without Yellow-winged Blackbird* *S. alterniflora* cover: $r^2 = 0.41$, $P < 0.05$).

4. Discussion

The concentration of ammonium, nitrate and phosphate and the percent of organic matter content was higher in marshes nearest to the sewage discharge source. Vegetation physiognomy, bird composition and abundance changed along a gradient of nutrient concentrations in a *S. alterniflora* marsh. Bird species and bird abundance increased near the nutrient discharge source. These changes were associated with the increase of height and cover of the *S. alterniflora*. The same pattern was recorded among height and cover of *S. alterniflora* with nutrient concentration. This study is one of the first that evaluates the relationship between nutrient enrichment and actual effects on higher trophic levels, such as birds.

The main effect of the discharge seems to be constrained relatively near to the nutrient source, given that nutrients and vegetation structure decreased abruptly, being approximately 50% lower within ~250 m of the sewage sourcewater. These patterns suggest that the *S. alterniflora* marsh is a 'sink' for anthropogenic nutrients (the concentration of ammonium decreased from 453 to 214 µM, phosphate from 442 to 221 µM, nitrate from 35 to 19 µM, the OM decreased from 15 to 9%), vegetation cover declined from 85 to 75%, and height dropped from 87 to 46 cm. These results suggest that perhaps *Spartina alterniflora* could be used as an ecological engineering organism to remediate eutrophicated salt marsh areas (Mitsch and Jørgensen, 1989; Marques et al., 2003).

The increase in habitat structure nearest the sewage outfall was exploited by bird species that use the neighboring interior and coastal habitats such as *S. densiflora* marsh, fresh marshes, upland shrubby habitats. These neighboring habitat species could take advantage of the increased refuge (e.g., species associated to dense grassland as Bay-capped Wren-Spinetail, *Spartonoica maluroides*; Cardoni et al., 2007; Mitchell et al., 2006), food (e.g., partially granivorous species as the Yellow-winged Blackbird; Darrieu et al., 2001) and nesting sites (Mitchell et al., 2006; Cardoni D.A. and Isacch J.P., unpublished data). On the other hand, the open areas generated by mat deposition were used mainly by waders and shorebirds at the Midway marsh, and by passerines and shorebirds in the marshes located near the sewage discharge outfall. The eutrophication at the Bahía Blanca marshes may, therefore, be producing a bottom-up effect that directly affects vegetation, and indirectly affects organisms higher in the trophic web, such as birds.

The species using marshes changed with seasons. The Bar-winged Cinclodes (*Cinclodes fuscus*) and Rufous-backed Negrito (*Lessonia rufa*) were found during winter, while the Bay-capped Wren-Spinetail and Grassland Yellow-Finch (*Sicalis luteola*) were recorded in the summer. Other birds, such as the Yellow-winged Blackbird, Correndera Pipit (*Anthus correndera*) and Chimango Caracara (*Milvago chimango*) were present all year. This pattern suggests that marshes with higher nutrient levels support the seasonal or annual requirements for different bird species, which would be using the salt marsh to nest, feed and/or rest.

The southern salt marshes from the SW Atlantic (Bahía Blanca, Bahía San Blas, San Antonio Oeste; Isacch et al., 2006) are dominated in the lower tidal elevation by monospecific grasslands of *S. alterniflora*, which have a relative short height and cover (Isacch et al., 2007), and are used occasionally by few bird species (Maldonado marsh in this study, Table 1; Isacch J.P., pers. obs.). Vegetation structure (e.g., height and cover) is an important factor for bird habitat selection, determining the bird abundance and richness in many different ecosystems around the world (Mac Arthur and Mac Arthur, 1961; Wiens, 1973; Roth, 1976). A number of bird species moves from adjacent habitats into the Bahía Blanca salt marsh when the structure changes in response to the increased nutrients. This is what happens with the Pale-breasted Spinetail (*Synallaxis albescens*) from shrublands, Bay-capped Wren-Spinetail, and Grass Wren (*Cistothorus platensis*) from the *S. densiflora* marsh, and Many-colored Rush-Tyrant (*Tachuris rubrigastra*), Wren-like Rushbird (*Phleocryptes melanops*) and Yellow-winged Blackbird from freshwater marshes. The Yellow-winged Blackbird was also recorded in low nutrient marshes, but the number of individuals was 18 times higher in the high nutrient marshes. Increased bird abundance and richness could not only be the result of vegetation structure, but also due to increased food availability. Nutrient enrichment has several effects on the salt marsh food web. It may increase the nutritive value of plants (protein, lipids, soluble carbohydrates in *S. alterniflora*; Biudes and Camargo, 2006), the abundance and biomass of

Table 1
Relative abundance of birds (total number of individuals) by species recorded in three areas with different nutrient levels in the Bahía Blanca saltmarsh, Argentina. Habitat association: TG: tall grassland; SG: short grassland; MF: mud flat. Bird-habitat association was based on studies of similar habitats in the region (Comparatore et al., 1996; Isacch and Martínez, 2001; Isacch et al., 2001, 2004; Cardoni et al., 2007).

	Naposta (High)	Midway (Intermediate)	Maldonado (Low)	Total	Habitat association
Passerines species					
Yellow-winged Blackbird, *Agelaius thilius*	1183	229	63	1475	TG
Correndera Pipit, *Anthus correndera*	56	21	4	81	SG
Bar-winged Cinclodes, *Cinclodes fuscus*	10	3	0	13	SG
Wren-like Rushbird, *Phleocryptes melanops*	13	0	0	13	TG
Rufous-backed Negrito, *Lessonia rufa*	1	10	0	11	SG
Great Pampa-Finch, *Embernagra platensis*	8	0	0	8	TG
Red-capped Wren-Spinetail, *Spartonoica maluroides*	7	0	0	7	TG
Many-colored Rush-Tyrant, *Tachuris rubrigastra*	6	0	0	6	TG
Pale-breasted Spinetail, *Synallaxis albescens*	5	0	0	5	TG
Grassland Yellow-Finch, *Sicalis luteola*	2	0	0	2	TG
Grass Wren, *Cistothorus platensis*	1	0	0	1	TG
Long-tailed Meadowlark, *Sturnella loyca*	0	1	0	1	TG
Raptors					
Milvago Chimango, *Caracara chimango*	175	68	25	268	SG,TG,MF
Long-winged Harrier, *Circus buffoni*	1	1	1	3	TG
Southern Crested-Caracara, *Caracara planctus*	1	1	0	2	SG,TG
Waders and shorebirds					
South America Stilt, *Himantopus melanurus*	4	122	0	126	MF
Southern Lapwing, *Vallenus chilensis*	3	8	0	11	SG,MF
Snowy Egret, *Egretta thula*	0	3	2	5	MF
Lesser Yellowlegs, *Tringa flavipes*	0	2	0	2	MF
TOTAL	1476	471	95	2040	

macroinfaunal species (Sardá et al., 1996), and the insect abundance (Bertness et al., 2008), thus causing indirect effects on the upper food web organism, such as birds and fishes (Raffaelli, 1999). Nutrient concentrations can affect birds differentially depending on the environmental characteristics or resources selected by each particular species or guild, such as benthic invertebrate-feeders (e.g., shorebirds, Correndera Pipit, Bar-winged Cinclodes), vegetation insect-feeders (e.g., Bay-capped Wren-Spinetail, Wren-like Rushbird), seed eaters (e.g., Yellow-winged Blackbird, Grassland Yellow-Finch, Great Pampa-Finch-*Embernagra platensis*) and generalist predators (Caracara Chimango). Our results show that salt marshes with nutrient enrichment had higher densities of *S. alterniflora* spikes than in salt marshes with relative low nutrient levels.

Table 2
Results of one-way ANOVA for total bird richness and abundance, abundance without Yellow-winged Blackbird (*Agelaius thilius*) and abundance of species associated to open gaps (see Table 1) for three areas with different nutrient levels in the *Spartina alterniflora* marsh from Bahía Blanca estuary, Argentina. For all comparisons degrees of freedom is 2.

	Total richness		Total abundance		Abundance without Yellow-winged Blackbird		Species associated to open gaps	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
July	7.3	0.007	32	0.00001	6.57	0.011	12.1	0.001
August	8.3	0.003	17.2	0.00007	6.69	0.007	6.4	0.008
September	5.5	0.01	5.7	0.01	5.10	0.018	1.7	0.2
November	2.8	0.09	12.2	0.0005	3.15	0.067	2.8	0.09
January	11.4	0.001	9.3	0.002	5.30	0.015	9.5	0.002
February	10	0.001	17.6	0.00006	2.80	0.048	9.1	0.002
March	5.6	0.01	8.6	0.002	1.50	0.25	10.2	0.1
April	14.1	0.0001	12.4	0.0004	1.82	0.19	10.2	0.001
June	7.5	0.004	11	0.0007	1.43	0.265	4.4	0.03

The increase in the abundance of seed-eater species, especially the Yellow-winged Blackbird, may result from the increment in the spike density of *S. alterniflora*. Reductions in the abundance of benthic infauna were observed close to a sewage output following an increase in sewage output (Hall et al., 1999). Some benthic invertebrates, however, notably annelids, are abundant close to sources of nutrient pollution (Green et al., 1990), favouring ground feeder birds. Increased arthropods in grass dominated habitats had been associated with increased complexity of plant architecture (Gibson et al., 1992; Dennis et al., 2001), and the increase of vegetation height (Morris and Plant, 1983; Dennis et al., 1997) and heterogeneity (Dennis et al., 1998; Vickery et al., 2001). Specifically, the number of herbivore insects increased when the nutritional quality of salt marsh plants improved as a result of higher nutrient loading increased (Vince et al., 1981). All of this evidence suggests that we might expect increased arthropod availability for birds with an increase of nutrient loadings to marshes. There is, therefore, an alternative possibility to explain why there are more birds in the high-nutrient marsh.

The dramatic increase of the raptor Chimango Caracara could be related to the increase of smaller birds and insects, two of the main prey of this species (Biondi et al., 2005). We suspect that Chimango Caracara is taking advantage of the disturbance, since the capacity and plasticity of this species to use new habitats have been demonstrated (Pedrana et al., 2008; Bellocq et al., 2008), including a remarkable ability to obtain food in novel situations (Biondi et al., 2008).

The association between increased seed production and the increase of seed-eater birds was also recorded in other SW Atlantic salt marsh, but was explained as a result of the indirect effect of the burrowing crab (*Neohelice granulata*) on *S. densiflora* marsh (Cardoni et al., 2007). This crab, because it builds caves, generates higher sediment water content and lower sediment hardness (Bortolus et al., 2004; Fanjul et al., 2007), and increases the nitrification-denitrification rate

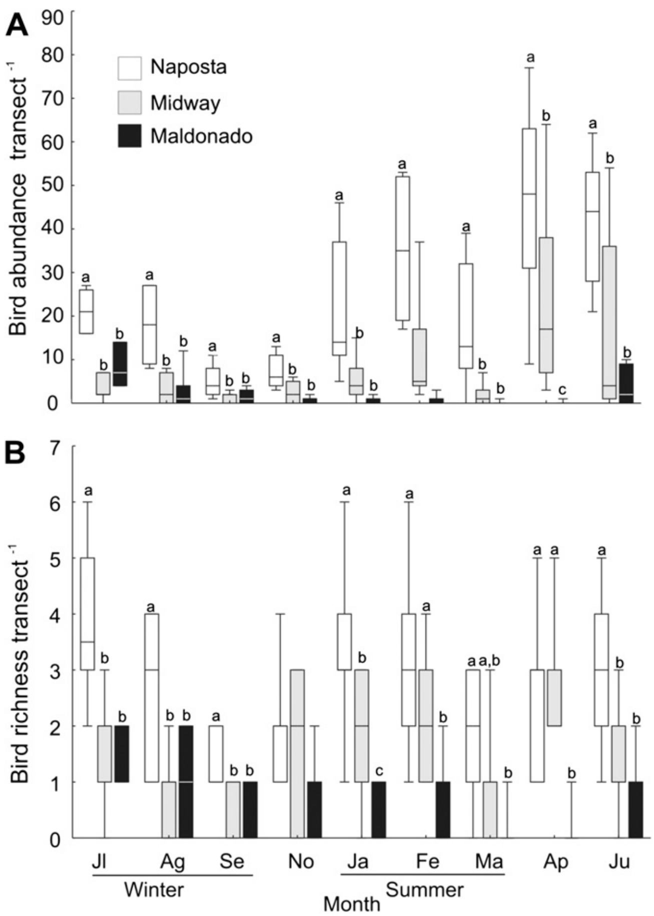

Fig. 6. Number of individuals (A), number of individuals without Yellow-winged Blackbird and species of birds (B) in three salt marshes at different distances from the sewage discharge (Naposta, Midway, Maldonado; see Fig. 1). The statistical data are in Table 1.

when soil oxygenation increases (Botto et al., 2005; Fanjul et al., 2007). The result is that plants allocate more energy to sexual reproduction when crabs are prevalent. Thus, different sources (i.e., natural and anthropogenic) of enhanced nutrient conditions (i.e., crabs caves and anthropogenic discharges) on different salt marsh plants may cause an increase of seed-eater bird species when compared with undisturbed salt marshes where insectivorous birds are the dominant trophic guild (Isacch et al., 2004; Cardoni et al., 2007).

Biomass generated by increased nutrient loading increases detrital production (Gonzalez Trilla et al., 2009) which also increases the generation of wracks. Marshes located close to the sewage showed higher number of wracks in the *S. alterniflora* marsh, generating higher landscape heterogeneity. Wracks, generate areas devoid of vegetation that are used by ground birds (e.g., Correndera Pipit, Bar-winged Cinclodes). Similar to the pattern recorded for the plant physiognomy, wracks may facilitate the use of the salt marsh by species from upper open habitats. This is a small scale disturbance, which is known to be important in maintaining species diversity (e.g. Grime, 1977; Grubb, 1977). Indeed, one of the well known cases is the effect produced by a tree fall in the rainforest, generating gaps that increase light penetration and therefore also increases plant diversity (Brawn et al., 2001). In our system, gaps produced by wracks generate habitat for open habitat bird species.

Salt marshes could be vulnerable if the nutrient delivery is discharged without management, given that it can ultimately degrade the structure of the marsh itself (Levine et al., 1998; Bertness et al., 2002; Crain, 2007). The sewage discharge into the Bahía Blanca estuary is being done without any treatment. In an advanced eutrophication process, the production of high amount of detritus could be generating a negative effect in marsh ecosystems (Hall et al., 1999) by several processes such as decreasing the local or regional biodiversity, increased competitive advantage of invasive species, loss of nutrient retention capacity or, shifts between "clear water" macrophyte-dominated systems to turbid phytoplankton-dominated systems (see EPA, 2008 for a review). The sewage effluent may also create conditions favoring parasites on fishes and wading birds (Coyner et al., 2002,2003). However, the growth of the aboveground biomass may be limited by nitrogen, but the belowground biomass may not be. The belowground biomass accumulation is what keeps the marsh at sea level and its biomass may be limited by phosphorus (Darby and Turner, 2008a,b). If the belowground biomass is compromised, then the long-term sustainability of the marsh may be harmed (Turner et al., 2009).

All the negative effects described above were not assessed in our study system, and we recognize that increases of some bird species, should be taken with caution and not as an overall beneficial effect of sewage discharges on the environment. Most bird species recorded in the eutrophicated marsh were common (Yellow-winged Blackbird) or extremely generalist species (Chimango caracara), without a specific conservation value. However, the movement of species into the eutrophicated marsh can turn in an ecological trap for ground or nearground nesting. *Spartina alterniflora* grows in the lower part of the intertidal, which in turn make nests vulnerable to frequent extreme floods by storms (Cardoni D.A and Isacch J.P. unpublished data).

In summary, our results show how local increments of nutrients generate direct and indirect changes in the salt marsh ecosystem. There are direct changes, as those caused on the vegetation physiognomy, and indirect changes in the composition and abundance of the bird assemblage.

Acknowledgments

This work was supported by grants and fellowships from ANPCYT, CONICET and Universidad Nacional de Mar del Plata to O. Iribarne.

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