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1 Relationship between anthropogenic sewage discharge, marsh structure and bird  
2 assemblages in a SW Atlantic saltmarsh

3

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5

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15 **Abstract.** One of the main effects of urbanization on coastal areas is through the  
16 discharge of sewage, which increases nutrient concentrations in the receiving  
17 environment. Salt marshes, like other coastal marine environments, are limited by  
18 nutrients, mainly nitrogen, and thus increasing nutrient loadings to a marsh may have  
19 consequences on marsh characteristics. We evaluated how the effects of nutrient  
20 enrichment in the form of sewage input, affected the vegetation structure and bird  
21 assemblages in a *Spartina alterniflora* salt marsh system near Bahía Blanca, Argentina  
22 (39° 01' S - 56° 25' W). Surveys of nutrient concentration, vegetation and birds were  
23 made at three different distances from the sewage discharge source. The concentration  
24 of ammonium, phosphate, and nitrate and the percent organic matter were higher in  
25 marshes nearest to the sewage discharge source. Bird composition and abundance, and  
26 vegetation physiognomy changed along a gradient of nutrient concentration. The  
27 increased habitat complexity found near the areas of higher nutrient concentration was  
28 exploited by birds that use neighboring interior and coastal habitats, including *Spartina*  
29 *densiflora* marshes, freshwater marshes and upland shrubby habitats. Our results show  
30 that local increases of nutrient inputs directly changed the vegetation physiognomy, and  
31 indirectly the composition and abundance of bird assemblages.

32

33 **Keywords:** Saltmarsh bird, *Spartina alterniflora*, eutrophication, coast, Bahía Blanca  
34 estuary, Argentina

## 35 1. Introduction

36 The human population tends to live near the coastline (Wolanski, 2007), and thus,  
37 human activities can have greater impacts on the coastal ecosystems than on inland  
38 habitats (UNEP, 2006; Valiela, 2006). One of the main results of coastal populations is  
39 the discharge of sewage, which involves increased nutrient concentrations in the coastal  
40 environment (GESAMP, 1990). Salt marshes, like other coastal ecosystems, have  
41 relatively low-nutrient supplies (Valiela and Teal, 1974), and so nutrient increases can  
42 have large consequences i.e., changing the (1) structural complexity of marsh plants by  
43 enhancing biomass and stem density, (2) species composition, and (3) zonation patterns  
44 (Levine et al., 1998; Daleo et al., 2008). Also, nutrient increases may lead to marsh  
45 subsidence and habitat fragmentation, depending on the balance between sediment  
46 depositions, increased plant production, and increased decomposition (Valiela and Teal,  
47 1974; Harper, 1995; Deegan, 2002). Specifically, in salt marshes characterized by large  
48 monospecific plant stands, nutrient enrichment may modify the plant physical structure  
49 by changing the plant growth form (Deegan, 2002). Nitrogen is usually the limiting  
50 nutrient controlling aboveground salt marsh vegetation production (Mendelssohn, 1979)  
51 and its availability increases the plant canopy and standing stocks, which is followed by  
52 higher rates of vegetation decay (Mitsch and Gosselink, 2000), and changes in species  
53 composition (Craft et al., 1995; Vaithyanathan and Richardson, 1997). A case largely  
54 studied in the northern hemisphere is the change of the dwarf form of *Spartina*  
55 *alterniflora* into the tall form as a result of an increase in nutrients loading to a marsh  
56 (Valiela and Teal, 1974; Valiela et al., 1978). This increased nitrogen loading, however  
57 may or may not lead to a net gain of the marsh depending on the balance between the  
58 change in marsh plant production and decomposition. Increases in the primary  
59 production or vegetation biomass may produce more detritus (Harper, 1995; Deegan,

60 2002) which is important in food webs (Deegan et al., 2000) and in creating peat that  
61 forms the physical structure of the marsh platform (Friedrichs and Perry, 2001). Dead  
62 vegetation deposition ('wracks') on coastal marshes is a natural disturbance that can  
63 increase habitat heterogeneity (Valiela and Rietsma, 1995; Minchinton, 2002). Thus  
64 when nutrients loadings to a salt marshes increase we expect greater vegetation  
65 heterogeneity because of the increased canopy structure and more frequent occurrence  
66 of wracks.

67 Vegetation structure, composition, and floristic characteristics are substantial factors  
68 influencing bird habitat selection, because these factors provide food, nesting sites, and  
69 cover from predators (Macnally, 1990; Vickery et al., 2001; Buchanan et al., 2006).  
70 Individuals select habitats that are an optimal combination of resources that allows them  
71 to perform multiple activities (e.g., foraging, breeding, roosting; Hilden, 1965; Fretwell  
72 and Lucas, 1970; Block and Brennan, 1993; Steele, 1993). Thus habitat structural  
73 heterogeneity is often a good predictor of bird diversity (Mac Arthur and Mac Arthur,  
74 1961; Wiens, 1973; Roth, 1976). This result is probably due to the role of habitat  
75 complexity in promoting niche diversification (Wilson, 1974; Roth, 1976). There are  
76 many examples of the effect of nutrient enrichment on the aquatic environment (i.e.,  
77 lakes estuaries; Robledano et al., 2008), such as those generated by algal blooms on  
78 waterbirds (Rosa et al., 2003), waterbird breeding (Rönkä et al., 2005) and shorebirds  
79 (Raffatelli 1999; Lopes et al., 2006). Bird abundance is positively correlated with lake  
80 nutrient levels (Nilsson and Nilsson, 1978; Hoyer and Canfield, 1990, 1994; McCarty,  
81 1997). However, although the effects of eutrophication are a well studied phenomena  
82 worldwide (Vitousek et al., 1997; Valiela, 2006), we are not aware of studies on the  
83 effects of nutrient enrichment on bird assemblages associated with salt marsh habitats.

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

96

## 97 2. Materials and Methods

### 98 2.1. Study area

99 The study was performed at the Bahía Blanca estuary (39°01'S-56°25'W;  
100 Argentina), which includes one of the largest SW Atlantic salt marshes (Isacch et al.,  
101 2006; Fig. 1). The bay has a total area of 2300 km<sup>2</sup> (Montesarchio and Lizasoain, 1981),  
102 with 10% of that surface covered by salt marshes and 20% by mudflats (Isacch et al.,  
103 2006). The salt marsh vegetation is dominated by two species; *Spartina alterniflora*  
104 grows in the lower marsh, and *Sarcocornia perennis* grows in the upper marsh. The  
105 industrial and port city of Bahía Blanca (300,000 inhabitants) is located on this coast.  
106 This city discharges its sewage into the bay relatively far from the city (Fig. 1). As in  
107 most of the bay, a large salt marsh dominated by *S. alterniflora* develops around the  
108 discharge point. Our observations suggested that there are strong variations in the salt

109 marsh vegetation characteristics linked with the sewage discharge, and that they are not  
110 typical of a natural gradient. The latter conclusion is supported by a study about  
111 biomass variation of *S. alterniflora* marshes developed in that region (Isacch et al.,  
112 2007), in which it was found that *S. alterniflora* plants reached larger sizes near the  
113 sewage discharge.

114 Three sites were selected at different distances from the sewage to study the  
115 relationship between anthropogenic nutrient concentration, the vegetation and birds,  
116 (Fig. 1). The salt marsh growing around the sewage discharge pipe and the proximal  
117 part of the discharge focus, were considered as the area of maximum nutrient effect  
118 (Naposta marsh, 10 ha; Fig 1). A second site located approximately 200 m from that  
119 source was considered as an area of medium effect (Midway marsh, 11.5 ha; Fig. 1). A  
120 third site located at 10000 m from the main discharge source was considered as an area  
121 of low effect (Maldonado marsh, 14.5 ha; Fig 1). All marshes have similar tidal  
122 conditions, given that they are positioned to the SW, and were located at the same  
123 distance (~1500 m) from the main tidal channel. We assumed, therefore, that the three  
124 marshes are similar except for their exposure to the sewage outfall.

125

## 126 2.2. Nutrient levels

127 To evaluate if the sewage discharge caused nutrient enrichment of the soil, we  
128 collected sediment samples to determine the total organic matter content (OM),  
129 concentration of dissolved inorganic nitrogen (nitrate and ammonium), and  
130 concentration of inorganic phosphorous (i.e., phosphate) in porewater. Twenty sediment  
131 samples were obtained using a stratified random sampling design at different distances  
132 from the sewage channel: 20 m and 80 m (Naposta marsh), 150 and 220 m (Midway  
133 marsh), and 10,000 m (Maldonado marsh). Sediment samples were collected by pushing

134 a PVC core (3 cm diameter, 8 cm length) into the sediment. Cores were immediately  
135 transported to the laboratory, where porewater was obtained by centrifugation ( $9000 \times$   
136  $g$ , 10 min), filtered, and stored at  $-20^{\circ}\text{C}$  until the analysis of dissolved nutrients. The  
137 nitrate concentration in porewater was determined calorimetrically as nitrite after  
138 reduction by cadmium followed by diazotization (Strickland and Parsons, 1968). The  
139 concentration of ammonium was measured using the blue indophenol method  
140 (Solórzano, 1969). The concentration of dissolved phosphate was measured using the  
141 molybdate method (Strickland and Parsons, 1968). A set of samples were dried (7 days  
142 at  $60^{\circ}\text{C}$ ) and combusted (8 h at  $500^{\circ}\text{C}$ ) to determine the total organic matter content  
143 (%) as the loss weight between dry and combusted sediment. The null hypothesis of no  
144 difference in nutrients (nitrate, ammonium and phosphate concentrations, and organic  
145 matter content) at different distances of the sewage discharge source were evaluated by  
146 using a one-way ANOVA. An *a posteriori* LSD Fischer test was used to identify  
147 differences when necessary (Zar, 1999). The relationship between nutrients  
148 concentrations and distance to the sewage discharge source was assessed by simple  
149 regression analysis (Zar, 1999).

150

### 151 2.3. Vegetation and bird surveys

152 We sampled vegetation and birds along 7 randomly distributed 100 x 60 m transects  
153 in each marsh using the fixed-width strip-transect method (following Bibby et al.,  
154 1997). Transects were separated by a minimum distance of 100 m. Birds were surveyed  
155 monthly between July 2007 and June 2008. The habitats included within transects were  
156 the vegetated marsh platform and the open gaps (determined by dead vegetation  
157 deposition, i.e. wracks). The vegetation physiognomy was surveyed in each bird  
158 sampling unit (i.e., transect) during winter and summer. In each transect used to survey

159 birds, we measured the following vegetation variables: height of the dominant strata of  
160 vegetation, plant species, percent of total and green coverage vegetation, and the  
161 number of *Spartina alterniflora* spikes (i.e., inflorescences). The vegetation data for  
162 each transect came from the average of two squares (1\*1 m) randomly located along  
163 transect. All vegetation surveys were made in vegetated areas. We assumed that the  
164 vegetation height and cover represented the main components of the habitat structure in  
165 marsh ecosystems (Cardoni et al., 2007).

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

175 There were dead vegetation depositions of *Spartina alterniflora* ('wracks') in areas  
176 closes to the sewage channel. To evaluate if marshes with different nutrient levels have  
177 a different density of wracks, we compared the number of wracks among them using the  
178 Google Earth software (<http://earth.google.com>). The study site was covered by an  
179 image from Google Earth with high resolution (date 01-12-2007) where wracks  
180 previously geopositioned were easily recognized. Then a portion of 10 ha in the core  
181 area was analyzed for each salt marsh (Naposta, Midway and Maldonado). Wracks in  
182 each image were digitized to determine the number and the cover area. The density of

183 wracks was then calculated in each case from the digitized images and differences  
184 among sites were evaluated using a Chi-square test (Zar, 1999).

185

### 186 3. Results

#### 187 3.1. Nutrient levels

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

194 Nutrient concentrations decreased with increasing distances from the sewage  
195 discharge source (Ammonium:  $r^2 = 0.34$ ,  $P < 0.05$ ; Phosphate:  $r^2 = 0.30$ ,  $P < 0.05$ ;  
196 Nitrate:  $r^2 = 0.47$ ,  $P < 0.05$ ) and no relationship was found between distance and OM ( $r^2$   
197  $= 0.17$ ,  $P > 0.05$ ).

198

#### 199 3.2. Vegetation

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

208 Naposta and Midway marshes than in the Maldonado marsh (ANOVA; winter:  $F_{2,18} =$   
209 9.92,  $P < 0.05$ ; summer:  $F_{2,18} = 8.95$ ,  $P < 0.05$ ; Fig. 4C).

210 The total and green cover of *S. alterniflora* were both higher in summer than winter  
211 at the three salt marshes (t-test; total cover, Naposta,  $t_{12} = 2.95$ ,  $P < 0.05$ ; Midway,  $t_{12} =$   
212 2.67,  $P < 0.05$ ; Maldonado:  $t_{12} = 5.18$ ,  $P < 0.05$ ; green cover, Naposta,  $t_{12} = 10.81$ ,  $P <$   
213 0.05; Midway,  $t_{12} = 4.31$ ,  $P < 0.001$ ; Maldonado,  $t_{12} = 8.01$ ,  $P < 0.05$ ). Vegetation height  
214 was better explained by nutrients concentrations (Phosphate,  $r^2 = 0.66$ ,  $P < 0.05$ ;  
215 Ammonium,  $r^2 = 0.70$ ,  $P < 0.05$ ; Nitrate,  $r^2 = 0.84$ ,  $P < 0.05$ , Fig. 5A) than vegetation  
216 cover (Phosphate,  $r^2 = 0.51$ ,  $P < 0.05$ ; Ammonium,  $r^2 = 0.51$ ,  $P < 0.05$ ; Nitrate,  $r^2 =$   
217 0.66,  $P < 0.05$ ).

218 There were 13 wracks (7.5 % of 10 ha marsh) at the Naposta marsh, 3 wracks (1.9  
219 % of 10 ha marsh) at the Midway marsh, and none at the Maldonado marsh (Chi-Square  
220 = 227.63,  $df = 1$ ,  $P < 0.05$ ).

221

### 222 3.3. Birds

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

233 The Yellow-winged Blackbird (*Agelaius thilius*) was the most abundant bird  
234 species, with 72 % of the total bird abundance. However, 80 % of the Yellow-winged  
235 Blackbird abundance was recorded only in the Naposta marsh. Other bird species  
236 associated with tall grass (without considering Yellow-winged Blackbird; Table 1) were  
237 only recorded at the Naposta marsh, the site with higher values of *S. alterniflora* cover  
238 and height. Bird species associated with open areas (i.e., short grass and mud flats;  
239 Table 1) had a higher abundance in the Naposta marsh than in the Midway and  
240 Maldonado marshes in four of ten months, and were higher in two months in the  
241 Naposta and Midway marshes than in Maldonado marsh (Table 2). It should be noted  
242 that there were more wracks in the Naposta marsh than in the others two marshes.

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

252

#### 253 4. Discussion

254 The concentration of ammonium, nitrate and phosphate and the percent of organic  
255 matter content were higher in marshes nearest to the sewage discharge source.  
256 Vegetation physiognomy, bird composition and abundance changed along a gradient of  
257 nutrient concentrations in a *S. alterniflora* marsh. Bird species and bird abundance

258 increased near the nutrient discharge source. These changes were associated with the  
259 increase of height and cover of the *S. alterniflora*. The same pattern was recorded  
260 among height and cover of *S. alterniflora* with nutrient concentration. This study is one  
261 of the first that evaluates the relationship between nutrient enrichment and actual effects  
262 on higher trophic levels, such as birds.

263 The main effect of the discharge seems to be constrained relatively near to the  
264 nutrient source, given that nutrients and vegetation structure decreased abruptly, being  
265 approximately 50 percent lower within ~250 meters of the sewage sourcewater. These  
266 patterns suggest that the *S. alterniflora* marsh is a 'sink' for anthropogenic nutrients (the  
267 concentration of ammonium decreased from 453 to 214  $\mu\text{M}$  for, phosphate from 442 to  
268 221  $\mu\text{M}$ , nitrate from 35 to 19  $\mu\text{M}$ , the OM decreased from 15 to 9%), vegetation cover  
269 declined from 85 to 75 %, and height dropped from 87 to 46 cm. These results suggest  
270 that perhaps *Spartina alterniflora* could be used as an ecological engineering organism  
271 to remediate eutrophicated salt marsh areas (Mitsch and Jørgensen, 1989; Marques et  
272 al., 2003).

273 The increase in habitat structure nearest the sewage outfall was exploited by bird  
274 species that use the neighboring interior and coastal habitats such as *S. densiflora*  
275 marsh, fresh marshes, upland shrubby habitats. These neighboring habitat species could  
276 take advantage of the increased refuge (e.g., species associated to dense grassland as  
277 Bay-capped Wren-Spintail, *Spartonoica maluroides*; Cardoni et al., 2007; Mitchell et  
278 al., 2006), food (e.g., partially granivorous species as the Yellow-winged Blackbird;  
279 Darrieu et al., 2001) and nesting sites (Mitchell et al., 2006; Cardoni D.A. and Isacch  
280 J.P., unpublished data). On the other hand, the open areas generated by mat deposition  
281 were used mainly by waders and shorebirds at the Midway marsh, and by passerines  
282 and shorebirds in the marshes located near the sewage discharge outfall. The

283 eutrophication at the Bahía Blanca marshes may, therefore, be producing a bottom-up  
284 effect that directly affects vegetation, and indirectly affects organisms higher in the  
285 trophic web, such as birds.

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

294 The southern salt marshes from the SW Atlantic (Bahía Blanca, Bahía San Blas, San  
295 Antonio Oeste; Isacch et al., 2006) are dominated in the lower tidal elevation by  
296 monospecific grasslands of *S. alterniflora*, which have a relative short height and cover  
297 (Isacch et al., 2007), and are used occasionally by few bird species (Maldonado marsh  
298 in this study, Table 1; Isacch J.P., pers. obs.). Vegetation structure (e.g., height and  
299 cover) is an important factor for bird habitat selection, determining the bird abundance  
300 and richness in many different ecosystems around the world (MacArthur and  
301 MacArthur, 1961; Wiens, 1973; Roth, 1976). A number of bird species move from  
302 adjacent habitats into the Bahía Blanca salt marsh when the structure changes in  
303 response to the increased nutrients. This is what happens with the Pale-breasted  
304 Spinetail (*Synallaxis albescens*) from shrublands, Bay-capped Wren-Spinetail, and  
305 Grass Wren (*Cistothorus platensis*) from the *S. densiflora* marsh, and Many-colored  
306 Rush-Tyrant (*Tachuris rubrigastra*), Wren-like Rushbird (*Phleocryptes melanops*) and  
307 Yellow-winged Blackbird from freshwater marshes. The Yellow-winged Blackbird was

308 also recorded in low nutrient marshes, but the number of individuals was 18 times  
309 higher in the high nutrient marshes. Increased bird abundance and richness could not  
310 only be the result of vegetation structure, but also due to increased food availability.  
311 Nutrient enrichment has several effects on the salt marsh food web. It may increase the  
312 nutritive value of plants (protein, lipids, soluble carbohydrates in *S. alterniflora*; Biudes  
313 and Camargo, 2006), the abundance and biomass of macroinfaunal species (Sardá et al.,  
314 1996), and the insect abundance (Bertness et al., 2008), thus causing indirect effects on  
315 the upper food web organism, such as birds and fishes (Raffaelli, 1999). Nutrient  
316 concentrations can affect birds differentially depending on the environmental  
317 characteristics or resources selected by each particular species or guild, such as benthic  
318 invertebrate-feeders (e.g., shorebirds, Correndera Pipit, Bar-winged Cinclodes),  
319 vegetation insect-feeders (e.g., Bay-capped Wren-Spintail, Wren-like Rushbird), seed  
320 eaters (e.g., Yellow-winged Blackbird, Grassland Yellow-Finch, Great Pampa-Finch,  
321 *Embernagra platensis*) and generalist predators (Caracara Chimango). Our results show  
322 that salt marshes with nutrient enrichment had higher densities of *S. alterniflora* spikes  
323 than in salt marshes with relative low nutrient levels. The increase in the abundance of  
324 seed-eater species, especially the Yellow-winged Blackbird, may result from the  
325 increment in the spike density of *S. alterniflora*. Reductions in the abundance of benthic  
326 infauna were observed close to a sewage output following an increase in sewage output  
327 (Hall et al., 1999). Some benthic invertebrates, however, notably annelids, are abundant  
328 close to sources of nutrient pollution (Green et al., 1990), favouring ground feeder birds.  
329 Increased arthropods in grass dominated habitats had been associated with increased  
330 complexity of plant architecture (Gibson et al., 1992; Dennis et al., 2001), and the  
331 increase of vegetation height (Morris and Plant, 1983; Dennis et al., 1997) and  
332 heterogeneity (Dennis et al. 1998; Vickery et al., 2001). Specifically, the number of

333 herbivore insects increased when the nutritional quality of salt marsh plants improved as  
334 a result of higher nutrient loading increased (Vince et al. 1981). All of this evidence  
335 suggests that we might expect increased arthropod availability for birds with an increase  
336 of nutrient loadings to marshes. There is, therefore, an alternative possibility to explain  
337 why there are more birds in the high-nutrient marsh.

338 The dramatic increase of the raptor Chimango Caracara could be related to the  
339 increase of smaller birds and insects, two of the main prey of this species (Biondi et al.,  
340 2005). We suspect that Chimango Caracara is taking advantage of the disturbance, since  
341 the capacity and plasticity of this species to use new habitats has been demonstrated  
342 (Pedrana et al., 2008; Bellocq et al., 2008), including a remarkable ability to obtain food  
343 in novel situations (Biondi et al., 2008).

344 The association between increased seed production and the increase of seed-eater  
345 birds was also recorded in other SW Atlantic salt marsh, but was explained as a result of  
346 the indirect effect of the burrowing crab (*Neohelice granulata*) on *S. densiflora* marsh  
347 (Cardoni et al., 2007). This crab, because it builds caves, generates higher sediment  
348 water content and lower sediment hardness (Bortolus et al., 2004; Fanjul et al., 2007),  
349 and increases the nitrification-denitrification rate when soil oxygenation increases  
350 (Botto et al., 2005; Fanjul et al., 2007). The result is that plants allocate more energy to  
351 sexual reproduction when crabs are prevalent. Thus, different sources (i.e., natural and  
352 anthropogenic) of enhanced nutrient conditions (i.e., crabs caves and anthropogenic  
353 discharges) on different salt marsh plants may cause an increase of seed-eater bird  
354 species when compared with undisturbed salt marshes where insectivorous birds are the  
355 dominant trophic guild (Isacch et al., 2004; Cardoni et al., 2007).

356 Biomass generated by increased nutrient loading increases detrital production  
357 (Gonzalez Trilla et al., 2009) which also increases the generation of wracks. Marshes

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

368 Salt marshes could be vulnerable if the nutrient delivery is discharged without  
369 management, given that it can ultimately degrade the structure of the marsh itself  
370 (Levine et al., 1998; Bertness et al., 2002; Crain, 2007). The sewage discharge into the  
371 Bahía Blanca estuary is being done without any treatment. In an advanced  
372 eutrophication process, the production of high amount of detritus could be generating a  
373 negative effect in marsh ecosystems (Hall et al., 1999) by several processes such as  
374 decreasing the local or regional biodiversity, increased competitive advantage of  
375 invasive species, loss of nutrient retention capacity or, shifts between “clear water”  
376 macrophyte-dominated systems to turbid phytoplankton-dominated systems (see EPA,  
377 2008 for a review). The sewage effluent may also create conditions favoring parasites  
378 on fishes and wading birds (Coyner et al., 2002, 2003). However, the growth of the  
379 aboveground biomass may be limited by nitrogen, but the belowground biomass may  
380 not be. The belowground biomass accumulation is what keeps the marsh at sea level  
381 and its biomass may be limited by phosphorus (Darby and Turner, 2008a,b). If the

382 belowground biomass is compromised, then the long-term sustainability of the marsh  
383 may be harmed (Turner et al., 2009).

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

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

397

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401

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### FIGURE CAPTIONS

Figure 1: Top: Bahía Blanca (Argentina) during a low tide. White dotted 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* saltmarshes from Bahía Blanca. The four main habitats identified here are uplands (white), saltmarshes (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 meters from the discharge source; and (C) the Maldonado marsh 10 Km from the discharge source.

Figure 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 ( $P < 0.05$ ).

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

Figure 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, Midway, Maldonado; see Fig. 1).

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

Figure 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.

Research highlights

Saltmarshes are increasingly affected by sewage discharge.

Nutrient changes the vegetation physiognomy, increasing the habitat complexity.

Bird composition and abundance increase in high nutrient saltmarshes.

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

<b>TOTAL</b>	1476	471	95	2040
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<sup>1</sup> 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).

	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

<sup>2</sup>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 bay, Argentina. For all comparisons degrees of freedom is 2.











