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ENVIRONMENTAL PROCESSES, WATER QUALITY DEGRADATION, AND DECLINE OF WATERBIRD POPULATIONS IN THE RIO CRUCES WETLAND, CHILE

Nelson A. Lagos[1], Pedro Paolini[2], Eduardo Jaramillo[3], Charlotte Lovengreen[4], Cristian Duarte[3], and Heraldo Contreras[3]

[1]*Centro de Investigación en Ciencias Ambientales (CIENCIA-UST), Universidad Santo Tomás*
Ejército 146
Santiago, Chile 650449
E-mail: nlagoss@ust.cl

[2]*Centro de Estudios Espaciales, Universidad de Chile*
Tupper 2007
Santiago, Chile 8370451

[3]*Instituto de Zoología*
Facultad de Ciencias, Universidad Austral de Chile
Independencia 64
Valdivia, Chile 657

[4]*Instituto de Física*
Facultad de Ciencias, Universidad Austral de Chile
Independencia 64
Valdivia, Chile 657

Abstract: Changes in wetland ecosystems may result from the interactions of endogenous processes with exogenous factors such as environmental fluctuations and anthropogenic influences. Since mid-2004, the Río Cruces wetland, a Ramsar site located in southern Chile (40°S), exhibited a sudden increase in mortality and emigration of the largest breeding population of Black-necked swans in the Neotropics, a massive demise of the dominant macrophyte *Egeria densa* (the main food of swans and several aquatic birds), and a seasonal appearance of turbid waters. We compared annual variation in rainfall, river flow, and radiation over the period 2000–2005 to assess the role of environmental factors on these wetland changes. Those factors, with the exception of a decrease in river flow during 2004, did not show significant inter-annual differences. However, when comparing Landsat images, we found in the visible and near-infrared spectrum, a corresponding increase and decrease in water reflectance for 2005 with respect to 2003 and 2001, respectively. These results may reflect the appearance of turbid waters and the decrease in cover of *E. densa*. All temporal changes were restricted to the northern and central zones of the wetland. In addition, spatial analysis showed a gradient in turbidity across the wetland waters, which was enhanced by estuarine influence during spring-tides. Censuses of aquatic birds (1999–2005) showed that only herbivorous birds exhibited a pronounced decrease in population abundance after mid-2004, while piscivorous birds continued normal cycling, with even some positive trends in abundance during 2004–2005. Population declines in herbivorous birds may be related to the demise of *E. densa* and suspension of sediments during periods of reduced river flow (2004) that gave rise to the turbidity in the wetland waters. Environmental changes could be related to changes in water quality after a new pulp mill, built upstream of the wetland, initiated operations during February 2004.

Key Words: aquatic birds, Landsat image, macrophytes, Ramsar site

INTRODUCTION

Biotic and abiotic changes in wetland ecosystems may emerge from complex interaction among environmental processes (van Bodegom et al. 2004). In general, changes in rainfall patterns can influence wetland hydrology by altering the timing and amount of atmospheric and ground-water inputs that may change water depth, solute concentrations, and temperature, all factors influencing important biotic properties of wetlands (Wilson and Keddy 1986, Gaudet and Keddy 1995, Weiher et al. 1996, Euliss et al. 2004). Additionally, water flow interacts with aquatic plants and influences the flushing of sediments and other chemical and physical characteristics of the water column (Leonard and Luther 1995, Rose and Crumpton 1996, Oldham and Sturman 2001). Biotic components associated with wetlands such as macrophytes (Engelhardt and Ritchie 2001) and avian assemblages (Jaksic 2004) are particularly sensitive to these abiotic changes, and several studies have showed the importance of interactions in animal and plant assemblages associated with wetland waters (Wilson and Keddy 1986, Gaudet and Keddy 1995, Weiher et al. 1996). However, due to increased pressure from human activities, the relative influences of environmental processes must be separated from anthropogenic influences in order to better understand the ultimate causes of biotic and abiotic changes in wetland ecosystems, and to implement management and restoration strategies in human-altered situations. However, disentangling environmental from anthropogenic causes in wetland changes is a major task that requires scientific information based on precise and rigorous sampling and experimental methodologies (Soto-Gamboa et al. 2007, Palma et al. 2008), which is rarely available. Alternatively, non-experimental approaches, such as long-term monitoring of ecological systems become useful tools for comparative studies designed to assess spatial and temporal scales of ecological patterns, and provide some clues about underlying processes that cause ecosystem-level changes (Lagos et al. 2005, Lagos et al. 2008).

The wetland of Rio Cruces, including Carlos Anwandter Nature Sanctuary, is located near the city of Valdivia in southern Chile (ca. 40°S, Figure 1). It was formed in May 1961, after the most intense earthquake ever recorded (9.5 on the Richter scale, Cisternas et al. 2005) when an ocean tsunami with a 10–15 m wave penetrated the estuarine areas around Valdivia. Following the subduction of an area of 4,877 ha, former agricultural and forest lands were flooded to form a new tidally influenced wetland ecosystem that was colonized by a diversity of aquatic plants and water birds (Ramírez et al. 1991, Schlatter et al. 1991, 2002). These ecological properties and their conservation value were internationally recognized when the wetland of Rio Cruces was designated as the first Neotropical Wetland of International Importance under the Ramsar Convention. However, since mid-2004, the wetland has experienced sudden biotic changes (Jaramillo et al. 2007) including: 1) mortality and massive emigration of aquatic birds, particularly the herbivorous Black necked swan, *Cygnus melacoryphus* Molina, which formerly used this wetland as its main breeding site in the Neotropical biogeographic region (Schlatter et al. 1991, 2002), 2) the massive disappearance of the waterweed *Egeria densa* Planch, the dominant submerged macrophyte of the ecosystem (Mulsow and Grandjean 2006, Jaramillo et al. 2007, Soto-Gamboa et al. 2007), and 3) the seasonal appearance of turbid waters within the wetland during the spring-summer season. The causes for these ecosystem-level changes were unknown.

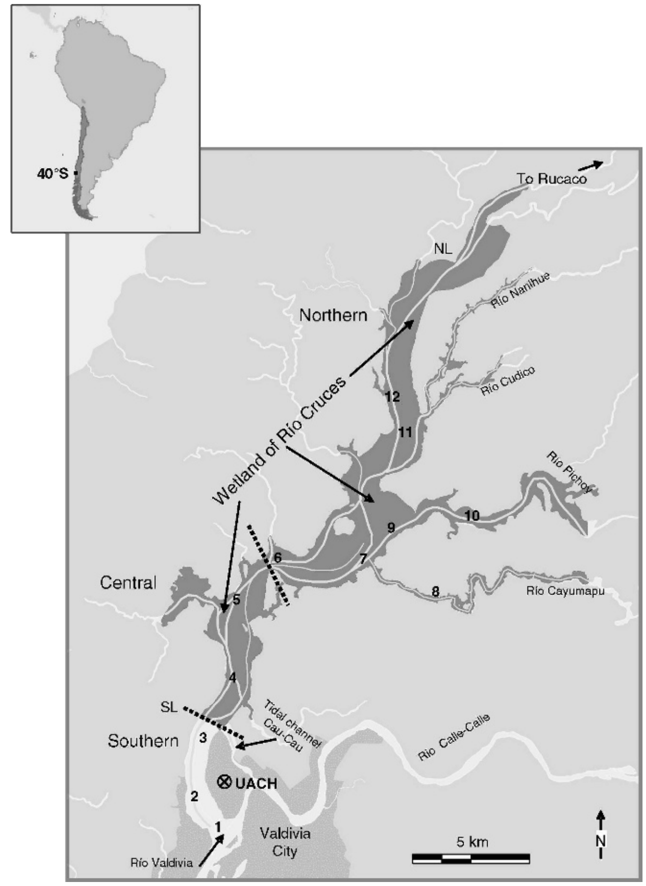

Figure 1. Geographic location of the Río Cruces wetland. The shaded areas are shallow water zones (swamps and marshes) that originated after the earthquake and tsunami of May 1960. NL and SL correspond to the Northern and Southern Limits of the Carlos Anwandter Nature Sanctuary. Numbers represent sampling stations for water quality assessment during neap and spring tides in March 2006. Dashed lines separate the northern, central, and southern zones analyzed for spectral reflectance profiles using Landsat images (see Methods for details).

In spite of the ecological importance of Rio Cruces wetland, no comprehensive ecological monitoring program was in place prior to the onset of environmental changes in 2004. A study by Ramirez et al. (1991) had described on the taxonomic status, distribution, and abundance patterns of aquatic macrophytes in the wetland. Schlatter et al. (1991, 2002) studied the reproductive behavior of Black necked swans and the relationship between the fluctuations of swan populations and environmental processes. Post-2004 studies have focused on the characterization of changes in the abiotic and biotic components of this wetland. Woelfl et al. (2006) investigated the concentration of heavy metals in plant remains of *E. densa* concluding that high concentrations of iron could explain its demise. Similarly, Mulsow and Grandjean (2006) concluded that a human-induced chemical disequilibrium in the waters of the wetland caused the demise of *E. densa* (but see Soto-Gamboa et al. 2007). As part of a large integrative effort to evaluate the environmental changes occurring within this Ramsar wetland site (UACH 2005), it was postulated that alteration in water quality occurred after a new pulp mill initiated operations during February 2004. However, and due to the lack of baseline scientific information, existing studies did not permit the establishment of precise cause-effect relationships concerning the ecosystem-level changes observed in the wetland of Rio Cruces (Palma et al. 2008). To remedy this situation, we performed a comparative study designed to characterize patterns of temporal and spatial variability in processes operating at a hierarchy of spatial scales. In particular, we worked 1) to identify patterns of temporal variation in rainfall, water flow, and radiation that operated at a regional scale and might have been associated with the abiotic and biotic changes observed in the wetland during 2004, 2) to characterize patterns of water reflectance using analyses of Landsat images that might be associated with spatial and temporal changes in water quality and aquatic plant cover within the wetland waters, 3) at a more local scale, to characterize patterns of spatial variation in water quality (turbidity and total suspended solids) across the wetland, and finally 4) to characterize intra-annual and long-term trends in the abundances of aquatic bird populations. Our study provides a synthesis of ecologically relevant environmental processes operating in the region, and is the first evaluation of changes in water quality of the wetland waters combining field and remote sensing data. Consequently, this information builds the current state of knowledge of the wetland of Rio Cruces that might be useful for ongoing efforts in planning, monitoring, and restoration for this threatened habitat.

METHODS

Study Area

The Rio Cruces originates in the pre-Andean Cordillera of southern Chile (ca. 39–40°S). It moves 170 km from east to west until meeting the tidal channel Cau Cau and the rivers Calle Calle and Valdivia (Figure 1). A 30 km portion of the river located north of the city of Valdivia corresponds to the wetland of Río Cruces and Carlos Anwandter Nature Sanctuary (Figure 1), and covers an area of 4,877 ha including many floodplain areas inundated to depths of almost 2 m. Most of the wetland is influenced by tides coming from the estuaries of the rivers Calle Calle and Valdivia. The wetland also receives inputs of fresh water from several tributaries (Nanihue, Cudico, Pichoy, and Cayumapu Rivers; Figure 1).

Regional Environmental Processes

Periodic data collected between 2000 and 2005 from the climatological station located at the main campus of Universidad Austral de Chile at Valdivia were used to characterize inter-annual variability in rainfall and photosynthetic active radiation (PAR) in the study area. Solar radiation was measured with a high resolution spectroradiometer (SUV-100, Biospherical Instruments Inc., San Diego, CA, USA) located on the roof (30 m above sea level) of the Faculty of Sciences building. Solar irradiance between 290 and 600 nm (a resolution of 0.6 nm wave band) was obtained in a daily scan lasting 15 minutes. During the scan a signal of PAR filter (400–700 nm), which was placed on the integrating sphere, was registered (see Huovinen et al. 2006). Daily maximum values of PAR were extracted and daily doses were calculated. We focus on the inter-annual variation (2000–2005) of PAR due to its potential impact on aquatic plant ecophysiology. River flow was measured over the same period at Rucaco station, located in the riparian area of the Rio Cruces (ca. 25 km upstream from the northern limit of the wetland, Figure 1).

Reflectance Data

One Landsat Thematic Mapper (TM) and two Enhanced Thematic Mapper (ETM+) images with Path/Row 233/88 of World Reference System 2 (WRS-2) with a central point near 40°19′20″ S, 72°51′00″ W were processed and analyzed (Table 1). All images corresponded to relatively cloudless days of the austral spring-summer period. Images were selected to contrast periods before and after the environmental changes observed during 2004

Table 1. Sensor, date information of the corresponding Landsat images studied and hours of the tidal stage at the time of the image acquisition. All images were acquired at Córdoba Station, Argentina. UTC = Universal Time Code. Tide hour state is referenced to the village of Corral, located at the estuary mouth of Rio Valdivia.

Sensor	Date – Hour	Tide High – Low
Landsat7/ ETM+	29-NOV-2001; 14:24 (UTC) 11:24 Summer Local Time	12:10–18:05
Landsat7/ ETM+	20-FEB-2003; 14:24 (UTC) 11:24 Summer Local Time	15:49–09:44
Landsat5/TM	1-FEB-2005; 14:15 (UTC) 11:15 Summer Local Time	18:06–11:33

(November 2001 and February 2003 versus February 2005). Although our focal year was 2004, no images for spring-summer were available for that year. The TM and ETM+ images were radiometrically calibrated and processed following equations and parameters described by Chander and Markham (2003). Briefly, digital numbers (DNs) of the image data were converted to spectral radiance (L_λ) values in W/(m^2·sr·µm units). Then, to compare images collected on different dates and by different sensors, L_λ values were converted to top-of-atmosphere reflectance (R_{TOA}) percentages. Furthermore, an atmospheric correction for Case-2 turbid waters was applied using the path extraction method (Hwan-Ahn et al. 2004) to account for absorption and scattering effects on water reflectance arising from diffusive radiation due to photons scattered by air molecules and aerosols, in addition to that being reflected at the water surface by total suspended solids (TSS), chlorophyll, and colored dissolved organic matter (CDOM). Basically, this correction is focused on extracting the path radiance (R_{path}) from the R_{TOA} signal by deleting the lowest radiance values in that band from the entire image. Path extraction assumes that in deep-blue waters the R_{TOA} signal is reduced to R_{path} because those waters have the lowest reflectance values (Antoine and Morel 1999), and that R_{path} is spatially homogeneous over the Landsat scene (see Hwan-Ahn et al. 2004 for corresponding equations and parameters). Finally, for each image, the value of reflectance was extracted from the corresponding 1 to 4 Landsat bands. Bands 1 to 3 correspond to visible ranges (0.42–0.52 µm, 0.52–060 µm, and 0.63–0.69 µm), while band 4 correspond to the near-infrared range (NIR) (0.76–0.90 µm). All image processing was performed using ENVI 4.1 (ITT Industries Inc., Boulder, CO, USA).

Rio Cruces wetland waters could be categorized into zones with different levels of change in water quality (see also Mulsow and Grandjean 2006). We selected three zones corresponding to the northern, central, and southern sections of the Rio Cruces wetland area (Figure 1), with spatial length of 15, 8, and 6 km, respectively (Figure 1). Incidental observations suggest that after 2004 and during the spring-summer season, brown colored waters occurred in the northern and central zones, and intermittently in the southern zone. This could result from estuarine tidal influences upon wetland waters. In the northern zone, reflectance values of the first four Landsat bands were extracted at 630 points within the area of the wetland waters. In the central and southern zones, reflectance values were extracted at 245 and 157 points, respectively. This process was repeated over each Landsat image and the corresponding spectral profile (i.e., plot of averaged reflectance versus Landsat bands) for each year and zone was constructed. As suggested by Parslow and Harris (1990) and Peñuelas et al. (1993), we used the blue/green ratio (Landsat bands 1 and 2), as a proxy of chlorophyll content in the water of the three zones studied. However, for the 2001 and 2003 periods, this surrogate measure did not distinguish between phytoplankton and submerged plants such as *E. densa* because both components were present simultaneously. For the 2005 image, the blue/green ratio in the northern and central zones (under constant presence of brown color waters) could be associated either to phytoplankton or to absorption by CDOM of water in the blue part of the spectrum. In addition, differences in tidal stage at the hour of the image acquisition may also influence the spectral properties of the image (see Table 1).

Field Data

We measured water transparency with a Secchi disk and took two surface water samples per station to analyze concentrations of TSS (after Strickland and Parsons 1972). To account for tidal effects on horizontal water transparency distributions and TSS, field samplings were carried out during spring and neap tides (March 14 and March 29, 2006, respectively). Sampling stations were situated as evenly spaced as possible northwards along the Rio Cruces and tributary rivers (Figure 1) with three stations in the southern zone, two stations in the central zone, and seven in the northern zone.

Waterbird Abundances

Monthly censuses carried out by the Corporación Nacional Forestal, Chile from January 1999 to September 2005 were used to analyze long-term and intra-annual trends in population abundances of six common waterbirds of the wetland. Three of them were herbivorous: *Cygnus melancoryphus* (Black necked swan), *Fulica armillata* Vieillot (Red gartered coot), and *F. leucoptera* Vieillot (small coot), and three were piscivorous: *Ardea cocoi* L. (Cocoi Heron), *Ardea alba* Gmelin (Great white egret), and *Egretta thula* Molina (Snowy egret).

Statistical Analyses

Pairwise comparisons and corresponding confidence intervals for inter-annual differences in rainfall, river flow, and PAR from 2000 to 2005 were assessed. Since the focal year was 2004, we tested if the values of daily measure for those environmental factors during this year were higher or lower than those measured during the same days in previous and subsequent years. We estimated the inter-annual difference as $\Delta_t = \text{variable}_{(t\,=\,2004)} - \text{variable}_{(t\,=\,\text{year}\ i)}$; where $\text{variable}_{(t\,=\,2004)}$ represent the value of the corresponding environmental factor recorded at the day *t* of the year 2004 and $\text{variable}_{(t\,=\,\text{year}\ i)}$ represent the value recorded in the same day in the year *i* (with *i* being the years 2000, 2001, 2002, 2003, or 2005). Because of multiple comparisons among years, the significance value was corrected using a Bonferroni approximation. All analyses were carried out using PROC GLM in SAS System 9.0 (SAS Institute Inc., Cary, NC, USA).

Statistical significance of the temporal variations in spectral reflectance profiles among years for each wetland zone was assessed using ANOVA followed by a Bonferroni multiple comparison test (PROC GLM). We use reflectance data recorded in each visible band and pooled them to characterize spatial and temporal patterns of water reflectance that may be associated with changes in water quality. We predicted an increase in reflectance (from increased turbidity) for the year 2005 in the northern and central zones. To assess temporal changes in water reflectance associated with changes in coverage of submerged macrophytes, we compared reflectance recorded in band 4 (NIR). In this case, we predicted a decrease in reflectance (from decreased macrophytes coverage) for the year 2005 in the northern and central zones of the wetland. We also tested for inter-annual differences in the blue/green ratio for each zone using ANOVA. In this case, we predicted a decrease in ratio (less chlorophyll content in water) for year 2005 across the whole study area. Tukey post-hoc comparisons were used to establish the rank of inter-annual differences in reflectance for each Landsat band. We tested for normality of residuals (Kolmogorov test) and for equal variance (Barlett's test), but did not find significant deviation ($P > 0.05$) from basic ANOVA assumptions.

To describe spatial trends in water quality parameters, a locally weighted scatter plot smoothing (LOWESS; see Lagos et al. 2005) was fitted to the spatial pattern in transparency and TSS as a function of the relative distance from Rio Valdivia (nearly 1 km south of station 1, Figure 1). This process was repeated for several values of the *f*-factor (or tension) to address the issue of how much smoothing is allowed due to subjectivity in selection. The *f*-factor selected was 0.5, indicating that 50% of the data was used to predict the LOWESS trend at the focal station. That factor produced normal LOWESS residuals and independence from geographical distance (Kolmogorov-Smirnov tests, $p > 0.05$). The analyses of LOWESS fit were carried out with MINITAB v13 (Minitab Inc., Pennsylvania, USA). To understand the spatial scale of water quality gradients under tidal influence, we estimated the Moran's *I* coefficients at five distance classes (Legendre and Legendre 1998) covering the full spatial extent of stations across the study area. Under the null hypothesis of no spatial autocorrelation, the expected value of *I* is zero, while positive and negative values, respectively, indicate positive and negative spatial autocorrelation in water transparency and TSS at the given distance class. The distance at which Moran's *I* value moves from positive to negative values is regarded as the characteristic spatial scale of the analyzed pattern (Lagos et al. 2005, 2008). Spatial autocorrelation analyses were carried out using SAAP 4.3 (Wartenberg 1989).

Population abundance analysis of waterbirds was based on the fitting of LOWESS trends as previously described, but in this case along time (Lagos et al. 2005). We fitted the LOWESS along all time periods to estimate the long-term trend (1999 to 2005) and trends within each year to estimate intra-annual patterns. The correlation between raw population abundance data and the LOWESS fit was significant in all cases. One-way ANOVA was used to evaluate differences in averaged population abundances among years. Prior to the analyses, data were $\log_{10}(x + 1)$ transformed to meet ANOVA assumptions.

RESULTS

Regional Environmental Processes

Over the period 2000–2005, rainfall was lowest in 2001 and highest in 2002 (Figure 2a). Mean annual rainfall in 2004 did not differ significantly from the annual means from 2000–2003 or 2005 (Table 2).

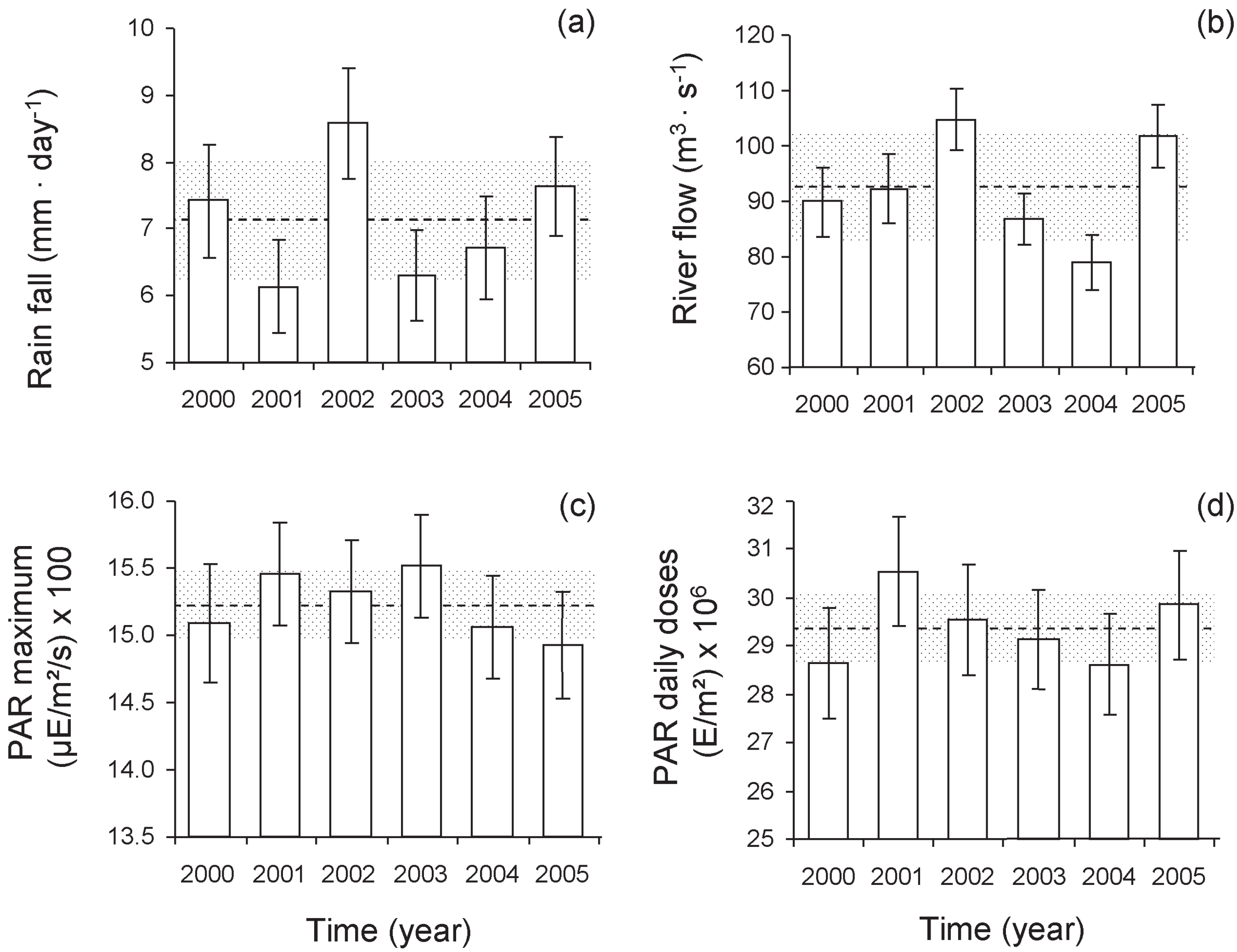

Figure 2. Temporal variation (annual mean ± 1 SD) for rainfall, river flow, and maximum and daily doses of PAR radiation. Dashed lines and shaded areas correspond to the mean and standard deviation for each environmental variable over the entire study period.

River flows were lowest during 2004 and highest during 2002 (Figure 2b). Mean annual river flow in 2004 was significantly lower than flows in 2002 and 2005 ($P < 0.001$, Table 2). Annual means of maximum PAR values recorded at Valdivia showed an increasing trend until 2003 and then a drop during 2004 and 2005 (Figure 2c), although these inter-annual variations were not significant after Bonferroni correction ($P > 0.01$, Table 2). Daily PAR doses were greatest during 2001 and then exhibited a decreasing trend until 2004 (Figure 2d), although only 2001 and 2004 differed significantly (Table 2).

Temporal Changes in Water Reflectance

As predicted, visible bands 2 and 3 in the northern and central zones of the study area showed a significant increase in reflectance in 2005 with respect to those found in images from 2001 and 2003 (Figure 3 and Table 3A). On the contrary, inter annual differences in reflectance patterns in the southern zone were distinct for each band; no statistical differences were found in inter-annual variations in reflectance measured in band 3 (Figure 3, Table 3A). Pooling reflectance values across visible bands highlight the increase in reflectance in the 2005 image with respect to that of 2003 and 2001, and in the northern and central zones. On the other hand, in the southern zone, differences in reflectance between the years 2005 and 2003 were significantly different, showing both years had higher reflectance compared to 2001 (Table 3B). In the northern and central zones, the NIR band showed a significant increase in water reflectance during 2003 with respect to the images of 2005 and

Table 2. Inter-annual pairwise comparisons in rainfall, river flow, and PAR recorded during the period 2000–2005. The focal year was 2004 because it corresponded to the year when sudden environmental changes were observed in the wetland of Río Cruces. Comparisons of PAR include only paired data available for January–February for 2005. Δ = averaged pairwise difference, i.e., Δ = variable $_{2004}$ − variable $_{\text{year (t)}}$; SD= standard deviation; CI = 95% confidence interval of Δ; t = t-statistic for the null hypothesis Ho: $\Delta = 0$. Due to multiple comparisons, significance was assessed using a Bonferroni approximation at $P < 0.01$ (in bold).

Variable	Pairwise Comparison	N	Δ	SD	CI	t	P-value
Rainfall (mm/d)	2004–2000	365	−0.68	20.7	(−2.81 ; 1.44)	−0.6	0.527
	2004–2001	365	0.59	18.6	(−1.32 ; 2.51)	0.6	0.542
	2004–2002	365	−1.85	21.0	(−4.01 ; 0.31)	−1.7	0.093
	2004–2003	365	0.43	18.1	(−1.43 ; 2.29)	0.5	0.648
	2004–2005	365	−0.90	18.8	(−2.84 ; 1.04)	−0.91	0.361
River flow (m^3/s)	2004–2000	365	−10.9	95.1	(−20.7 ; 1.1)	−2.19	0.030
	2004–2001	365	−13.1	105.8	(−24.1 ; 2.3)	−2.38	0.018
	2004–2002	365	−25.6	129.1	(−38.9 ; −12.4)	−3.8	< **0.001**
	2004–2003	365	−7.6	69.6	(−14.8 ; −0.5)	−2.09	0.037
	2004–2005	365	−21.8	89.9	(−31.1 ; −12.5)	−4.61	< **0.001**
PAR Maximum ($\mu E/m^2/s$)	2004–2000	298	15.5	35.4	(−54.1 ; 85.2)	0.4	0.661
	2004–2001	359	−17.1	30.2	(−76.5 ; 42.4)	−0.6	0.573
	2004–2002	303	73.3	36.9	(0.7 ; 145.9)	2.0	0.048
	2004–2003	362	−54.1	30.8	(−114.7 ; 6.4)	−1.8	0.080
	2004–2005	59	36.7	50.5	(−64.4 ; 137.8)	0.7	0.470
PAR daily doses (E/m^2)	2004–2000	298	0.73	1.00	(−1.24 ; 2.71)	0.7	0.464
	2004–2001	359	−1.23	0.87	(−2.971 ; 0.516)	−1.4	0.167
	2004–2002	303	1.18	0.98	(−0.743 ; 3.105)	1.2	0.228
	2004–2003	362	−0.91	0.84	(−2.559 ; 0.738)	−1.1	0.278
	2004–2005	59	2.96	2.26	(−1.57 ; 7.48)	1.3	0.196

2001. In the southern zone, differences between 2003 and 2005 did not reach statistical significance, but both years had higher water reflectance as compared to 2001 (Figure 3 and Table 3C).

Blue to green bands ratio, used as a proxy measurement of chlorophyll content in the water, showed that until 2003, high values occurred in the northern zone with decreasing levels towards the central and southern zones. During February 2005, the three zones showed an inverse pattern of blue/green ratios values (Figure 4). Results of ANOVA indicated significant inter-annual differences in the blue/green ratio in each zone (Table 3D). In addition, the coefficient of variation in the blue/green ratio was lower for the image of February 2005, suggesting a systematic decrease in spatial variation in chlorophyll water content among wetland zones (Figure 4).

Spatial Trends in Water Transparency and TSS

During the neap-tide, the distribution of water transparency was very similar across most wetland waters, with a maximum Secchi disk depth of *ca.* 150 cm. However, the southernmost station sampled located at the Rio Valdivia had a Secchi disk depth close to 220 cm (Figure 5a). During the neap-tide, no significant spatial trend in TSS across stations was observed (Figure 5c), and no significant spatial gradient was evident in correlograms constructed for transparency (Moran's I = 0.55, P = 0.204, Figure 5e) and TSS (Moran's I = 0.130, P = 0.578, Figure 5g). In contrast, during the spring-tide sampling period, a gradual decrease in water transparency was observed as distance upstream of the wetlands increased (Figure 5b). Concentrations of TSS increased across the same gradient (Figure 5d). The spatial correlograms estimated for samples gathered during the spring-tide period showed that transparency (Moran's $I = -0.92$, P < 0.001, Figure 5f) and TSS (Moran's $I = -0.98$, P < 0.001, Figure 5h) exhibited significant spatial structure, yielding positive spatial autocorrelation values among stations located at short distances classes (< 10 km) and a decrease to negative autocorrelation as distance classes increased among stations. Thus, stations located at distances < 10 km were more similar in transparency and TSS than expected by chance. Further, the spatial gradient in water quality showed a characteristic spatial scale of about 10 km (arrows in Figures 5f and 2h), which is roughly the averaged spatial extent of the categorized zones of the wetland (mean ± 1 SD = 9.6 ± 4.7 km, Figure 1) that was used for water reflectance

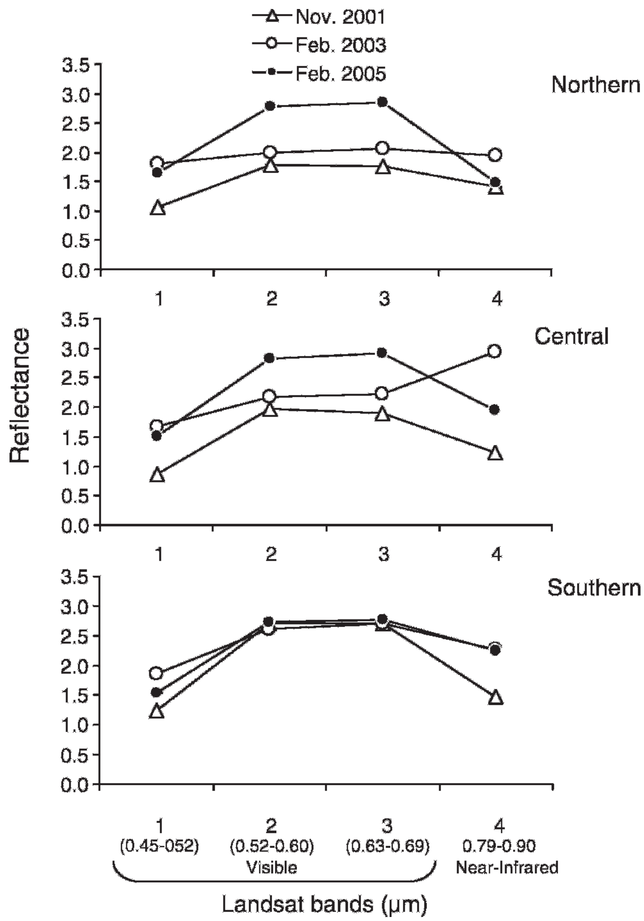

Figure 3. Spectral reflectance profiles (mean ± 1 SD, not visible due to minimal variation) for the three categorized zones of the Rio Cruces wetland waters and for the years of the Landsat images analyzed. Visible bands (1 to 3) were used to test for inter-annual differences in reflectance associated to changes in water transparency. NIR band (4) was used to test for inter-annual differences in reflectance associated with changes in aquatic submerged macrophyte coverage.

assessment (see above). The water transparency and TSS showed a positive and significant relationship (Pearson $r = 0.67$; $P < 0.001$; $n = 21$), which did not change during the neap tide (Pearson $r = 0.56$; $P < 0.036$) nor spring tide (Pearson $r = 0.86$; $P < 0.001$) periods.

Temporal Variability in Abundance of Waterbirds

Temporal trends (LOWESS) fitted to long-term and intra-annual population abundances of herbivorous and piscivorous waterbirds highlighted qualitative and quantitative differences between both functional groups (Figure 6). In general, long-term trends of herbivorous species (Black necked swans and coots) showed abrupt changes from the first trimester of 2004, declining to only a few hundred individuals by 2005 (Figure 6). The results of ANOVA show significant inter-annual differences in population abundance for each of the three herbivorous species (*C. melancoryphus*: $F_{6,74} = 88.44$, $P < 0.0001$; *F. armillata*: $F_{6,74} = 110.8$, $P < 0.0001$; *F. leucoptera*: $F_{6,74} = 4.78$, $P < 0.0001$), and Tukey post-hoc comparisons show that lower population abundances occurred during 2005 (Figure 6). On the other hand, long-term trends in population abundances of the piscivorous species (mostly herons) remained almost unaltered over the study period. Abundances of *A. cocoi* were higher in 2004 ($F_{6,74)} = 2.41$, $P = 0.035$), but abundances of *A. alba* and *E. thula* did not vary among years ($P > 0.05$).

DISCUSSION

Several studies have shown that environmental variation can influence ecological community composition. Thus, wetland ecosystems are reactive to environmentally driven changes affecting their structure and function (Wilson and Keddy 1986, Gaudet and Keddy 1995, Weiher et al. 1996). However, in this study we found that, with the exception of river flow, temporal variations in environmental factors operating at regional scales (UV radiation and PAR) were within the normal historical conditions for the study area (see also Huovinen et al. 2006).

Pezzato and Camargo (2004) found that in Brazilian rivers gross photosynthesis of *E. densa* was highest value of when underwater PAR was 895–1,126 µmol/m/s, and a positive relationship existed between submersed PAR and photosynthetic rate. Thus, we would expect a similar relationship in the wetland of Rio Cruces and conclude that the consistent temporal patterns in PAR variation over the period 2000–2005 cannot explain the sudden and massive die off of *E. densa* during mid-2004. On the other hand, significant reductions in river flows, such as those detected for the Río Cruces during 2004 may well influence the hydrological regime, a recognized key driver in all wetland ecosystems (Keddy and Fraser 2000, Mitsch and Gosselink 2000). A reduction in rainfall would exacerbate the effects of reduced river flow. In that scenario, discharge of wetland waters may dominate the recharge process, altering hydroperiod, and in turn, ecosystem functioning. Childers et al. (2006) report that hydroperiods in estuarine wetlands decrease during dry seasons associated with ENSO events, and that large scale disruption may alter wetland functioning. In southern Chile, the ENSO/Southern Oscillation Index was negative from 2001 through 2005 indicating an extended period of dry conditions

Table 3. Results of one-way ANOVA and Tukey post hoc test analyses carried out to compare temporal variation in the reflectance data for the three zones of the study area. Proximal analyses for turbidity compare reflectance among years using separate (A) and pooled (B) visible Landsat bands. Reflectance by the aquatic submerged plants was assessed by using near infrared bands (C) while water content of chlorophyll was estimated through the use of the blue/green ratio. Due to the multiple comparisons in the Tukey test analyses, significant differences between years were corrected at the indicated critical P value. Asterisks indicate rejection or acceptance of the underlying null hypothesis, in agreement with our working hypotheses.

Feature Analyzed	Zone	Landsat Band	DF (Source, Error)	MS	F	P-value	Tukey post hoc Test (P critical < 0.0195)
(A) Turbidity (individual bands)							
	Northern	1	2, 1858	0.00955	2106.0	0.0001	2003 > 2005 > 2001
		2	2, 1858	0.01726	1531.4	0.0001	2005 > 2003 > 2001*
		3	2, 1858	0.01985	986.1	0.0001	2005 > 2003 > 2001*
	Central	1	2, 732	0.00434	984.0	0.0001	2003 > 2005 > 2001
		2	2, 732	0.00474	359.8	0.0001	2005 > 2003 > 2001*
		3	2, 732	0.00653	278.2	0.0001	2005 > 2003 > 2001*
	Southern	1	2, 468	0.00158	340.9	0.0001	2003 > 2005 > 2001
		2	2, 468	0.00005	5.3	0.005	2005 = 2001 > 2003*
		3	2, 468	0.00002	0.7	0.484	2005 = 2003 = 2001*
(B) Turbidity (pooled bands)							
	Northern	1, 2, 3	2, 5582	0.0371	1415.9	0.0001	2005 > 2003 > 2001*
	Central	1, 2, 3	2, 2202	0.01265	334.9	0.0001	2005 > 2003 > 2001*
	Southern	1, 2, 3	2, 1410	0.00040	8.5	0.0001	2005 = 2003 > 2001*
(C) Aquatic plant cover							
	Northern	4	2, 1858	0.00524	38.3	0.0001	2003 > 2005 = 2001*
	Central	4	2, 732	0.01822	67.7	0.0001	2003 > 2005 > 2001*
	Southern	4	2, 468	0.00338	8.3	0.0001	2003 = 2005 > 2001*
(D) Chlorophyll content in water							
	Northern	1/2	2, 1858	27.0807	1876.8	0.0001	2003 > 2005 = 2001*
	Central	1/2	2, 732	6.1207	846.0	0.0001	2003 > 2005 > 2001*
	Southern	1/2	2, 468	2.2749	558.5	0.0001	2003 > 2005 > 2001*

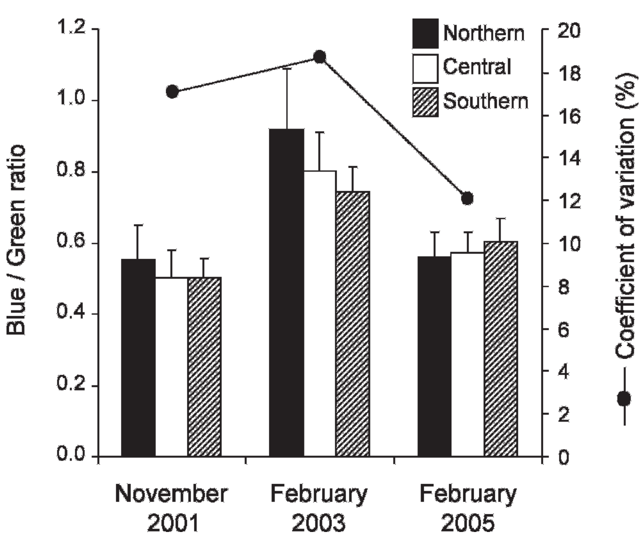

Figure 4. Spatial and temporal variation (mean ± 1 SD) in the blue/green ratio (Band 1/Band 2) as a proxy for chlorophyll content in the water. The coefficient of variation in the ratio values among zones is showed in black dots and scaled on the right axis.

(NOAA Climate Prediction Center, www.cpc.ncep.noaa.gov).

Remote sensing studies indicated that patterns of spectral reflectance of waters were mainly associated with changes in turbidity produced by TSS. In general, low spectral reflectance was linked with zones having relatively low concentrations of TSS and vice versa. This was especially true for values around 0.55 μm; at that wavelength region there is a good match between upwelling radiance and TSS, either in fresh or marine waters (Munday and Zudkoff 1981). Additionally, a positive and quasi-linear dependence is believed to exist between TSS in the range of 0 to 50 mg·L^{-1} and spectral responses in the range of 0.4–0.9 μm (Curran and Novo 1988). TSS levels in the waters of the Río Cruces wetland were within this range suggesting a positive and direct relationship with the spectral reflectance in visible bands of the Landsat image. Our analyses of Landsat images showed that during February 2005, the wetland waters presented higher reflectance in the visible range as compared to November 2001

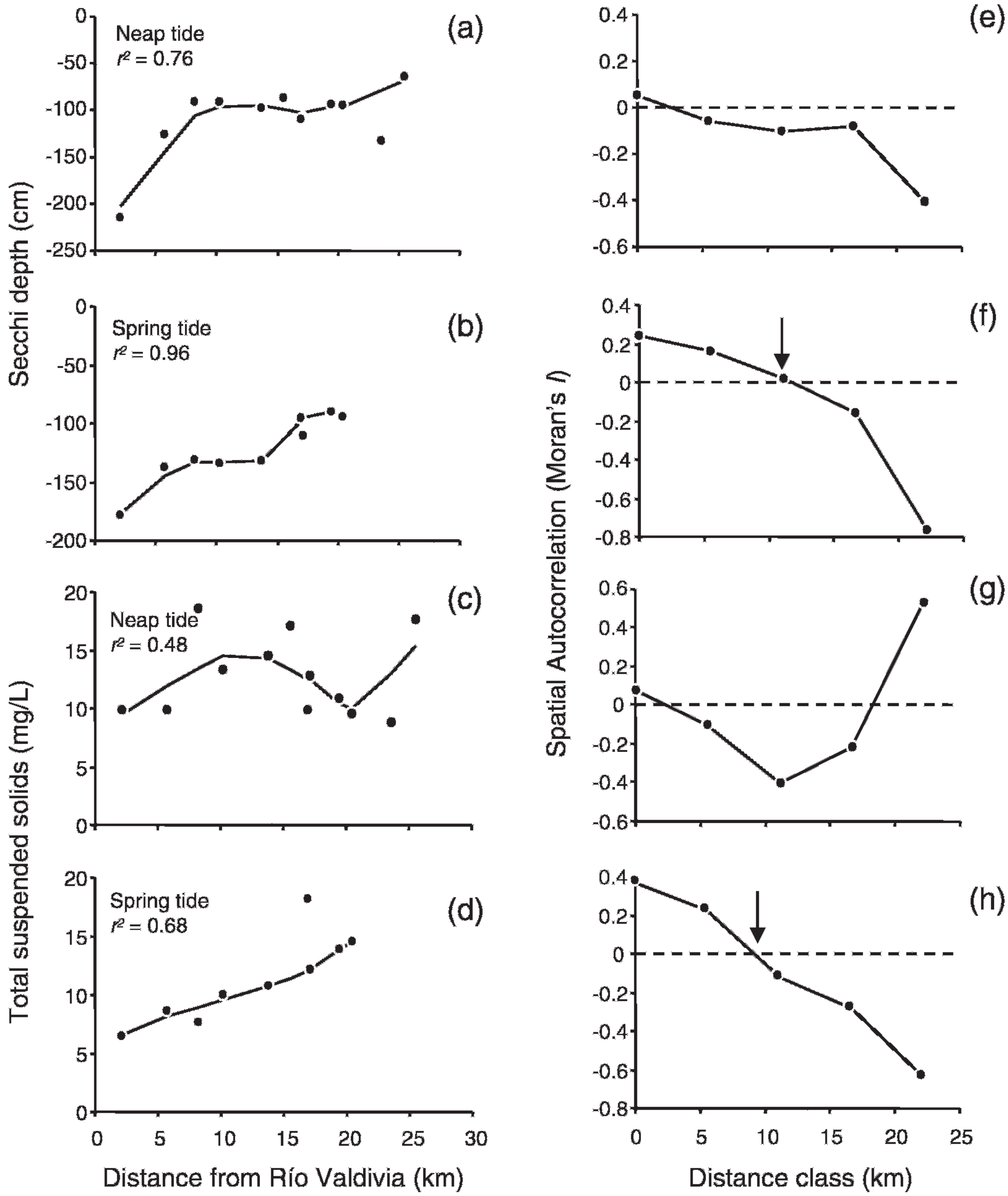

Figure 5. Spatial trends of transparency and concentrations of total suspended solids along the wetland of Rio Cruces and tributary rivers. Panels on the left side correspond to the adjustment of the fitted LOWESS for transparency (a–b) and total suspended solids (c–d) during the neap and spring tides of March 2006. Panels on the right side correspond to the Moran's *I* spatial correlograms for the corresponding variables (e–h); the values of Moran's *I* index and significance for the overall correlogram are shown. The arrows on the correlograms indicate the characteristic scale of the spatial gradient in the distribution of transparency and total suspended solid across the area.

and February 2003, which may correspond to the increase in turbidity in the northern and central zones due to a higher load of TSS (see also Jaramillo et al. 2007).

Studies designed to characterize the spectral properties of waters with variable phytoplankton concentrations and aquatic macrophytes (e.g., Peñuelas et al. 1993) suggest that chlorophyll in water is indicated by maximum spectral reflectance between 0.55 μm (green) and 0.70 μm (NIR) (Han 1997). Thus, lower reflectance at the NIR range found in February 2005, as compared to 2003 and 2001, may be interpreted as a reduction in primary productivity associated with the loss in cover of the aquatic plant *E. densa* in the northern and central zones of the Río Cruces wetland. However, the influence over the water reflectance by differences in spectral sensitivity between Landsat 5 TM and

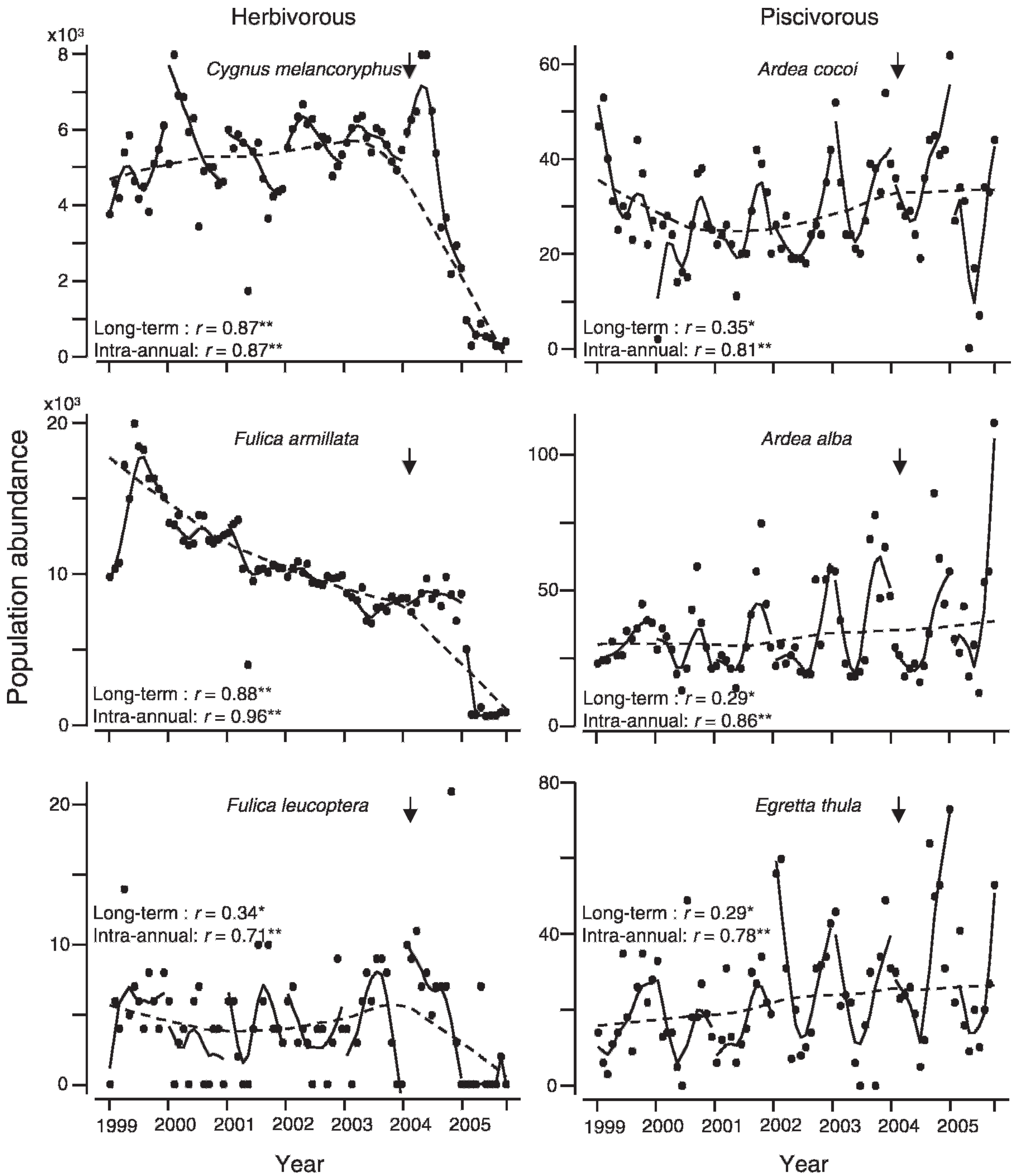

Figure 6. Patterns of temporal variability in the population abundance of selected herbivorous and piscivorous birds inhabiting the wetland of Rio Cruces. Dashed line = long-term trend; solid line = intra-annual trend for the corresponding year. In all cases, trends correspond to a LOWESS fitted over the corresponding temporal scale. The arrows indicate the start of discharges of the pulp mill effluent into the waters of Rio Cruces. r = Pearson correlation between raw data (black dots) with the fitted values (lines) at the corresponding temporal scale. * $P < 0.001$; ** $P < 0.0001$.

Landsat 7 ETM for band 4 cannot be ruled out (Teillet et al. 2007). Additionally, the values of the blue/green algorithm (Parslow and Harris 1990) used to estimate concentrations of chlorophyll in this wetland, suggest a reduction of spatial heterogeneity in environmental conditions across the area. A decrease in primary productivity in the wetland of Rio Cruces could be the result of a decrease in the cover of *E. densa* and a corresponding replacement by other suspended materials (see Jaramillo et al. 2007), which may include phytoplankton. However, our remote sensing process cannot confirm this. Although changes may represent alternative stable states operating in the aquatic ecosystem of Rio

Cruces, a lack of studies focused on temporal variation of phytoplankton and macrophyte assemblages and their relative roles in productivity preclude further interpretation.

Higher turbidity and higher loads of TSS within the wetland (northern and central zones of the study area) may be related to the massive reduction in cover of *E. densa*, resulting in flushing of sediments from shallow floodplain areas during periods of high spring tides and low river levels. High turbidity and changes in reflectance patterns remained localized within the northern and central zones of the wetland, while waters in the southern zone of the study area, which were more affected by tidal flushing, did not exhibit significant changes in terms of reflectance patterns. Field and remote sensing data analyzed in this study reveal that the current state of water quality across the study area had a spatially structured gradient, not evident in images prior to 2004. Earlier, *E. densa* covered large portions of the wetland, especially shallow areas (Ramirez et al. 1991); this entrapped suspended materials and improved water quality across the wetland. The sudden disappearance of *E. densa* in mid-2004 probably contributed to the deterioration in water quality conditions of the wetland of Rio Cruces and its continued absence may perpetuate this state (Jaramillo et al 2007). During the austral spring-summer seasons of 2004 to 2008, brown colored waters have been observed moving downstream from the wetland and into clearer waters of the southern zone, a situation reinforced by simultaneous decreases in river flow (see www.humedalriocruces.com). Finally, environmental change could also be influenced by tidal stage. Images for 2003 and 2005 were influenced by low tide, while the image for 2001 was more influenced by high tide (Table 1). As *E. densa* is a submerged plant, water levels over plants may affect the attenuation by water absorption in the NIR band of 2001 image. High tide conditions might constrain turbid water to the northern zone of the wetland. However, spectral patterns were consistent for the three images used, suggesting persistent clear water and cover of macrophytes in the southern zone. As such, differences in water reflectance found in this study seem to be more determined by annual differences in spectral properties of wetland water rather than any influence of tidal stage.

Changes in the waterbird community were restricted to herbivorous birds (swans and coot) that foraged primarily on *E. densa* (Corti and Schlatter 2002). The lack of response by piscivorous herons suggests that environmental changes in 2004 did not propagate to all biotic components. This suggests that herbivorous bird populations might rebound if *E. densa* recovers.

Our study provides some clues about possible causes for the observed biotic and abiotic changes occurring in the Rio Cruces wetland. It is unlikely that the massive die off of *E. densa* and the seasonal presence of brown color waters after 2004 (Woelfl et al. 2006, Jaramillo et al. 2007, Soto-Gamboa et al. 2007) resulted from regional environmental process operating at spatial scales larger than the river basin. On the contrary, our results indicate that environmental changes were operating at smaller spatial scales, within the wetland and only in the northern and central zones. As far as we know, the only process operating in that scale was the human-induced change in water quality that occurred after a pulp mill started operations in February 2004 and introduced effluents into the Rio Cruces 25 km upstream the wetland (UACH 2005, Mulsow and Grandean 2006, Jaramillo et al. 2007, Soto-Gamboa et al. 2007). On several occasions during 2004, pulp mill effluents exceeded the authorized level of 50 mg/L for suspended solids (see www.conama.cl and www.e.seia.cl). In addition, large amounts of aluminum sulphate are used in the chemical treatment of the liquid wastes of the pulp mill to coagulate particles, and levels of sulphate and aluminum have increased downstream of the mill (UACH 2005, Mulsow and Grandjean 2006). However, more studies, including spatio-temporal variability of environmental factors and its interactions with biological variables are needed to identify the ultimate causes of ecosystem-level changes in the Rio Cruces wetland. Such information will provide a solid basis for future management and conservation strategies to restore ecosystem services of this Ramsar site in the Valdivian Forest of Southern Chile.

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