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INTRODUCTION

Ecosystem services and the function of wetlands are increasingly altered by human actions around the world. Wetland ecosystems are scientifically and socially important because they represent high-biodiversity areas and water reservoirs. In southern Chile (ca. 39–41°S), there are several coastal and inland wetlands larger than 500 ha that are currently threatened by adjacent urban locations due to the discharges of industrial wastes, conversion of soil to agriculture, and water extraction. These Chilean wetlands are mostly located on the coastal range of the Valdivian Rainy forest, which is biologically unique due to its high plant diversity and is also being affected by fragmentation resulting from extensive land use changes. These characteristics of the wetland and Valdivian forest have been the promoters of national and international efforts that highlight the need for conservation of these ecosystems (1).

Here we describe recent significant biotic changes observed in the Ramsar wetland site of Río Cruces, a nature sanctuary under Chilean law and located north of Valdivia city (ca. 40°S) (Fig. 1). The site originated during May 1960 as a land subsidence from the worst earthquake recorded in history (9.5° on the Richter scale) and ensuing tsunami with wave crests reaching 10 to 15 m (2), flooding a present area of more than 5000 ha mostly composed of shallow water zones represented by swamps and marsh vegetation. This tectonically originated Ramsar site is also unique, because it became the main reproductive site of the charismatic and vulnerable Black-necked swans (*Cygnus melancoryphus*) in the neotropical area of South America (3–5). Apart from that uniqueness, the high diversity of water birds and aquatic plants (6, 7) was also pointed out by the Chilean government, when this site was designated a Nature Sanctuary (3 June 1981). The sanctuary was then accepted as the first Neotropical Wetland of International Importance under the Ramsar Convention on 27 July 1981.

HISTORICAL BRIEF

C. melancoryphus (Anatidae) is the only representative of the genus *Cygnus* native

to the neotropical region (8–10), with its showy presence and abundance becoming a charismatic species and indicator of relative health of the wetlands, such as the Río Cruces Ramsar site (11, 12). During the late 1980s (1987), the annual mean swan population abundance increased from nearly 1400–2200 to 6100–6300 individuals during the mid 1990s (1995–1996), abundances that remained fairly constant until 2003 (Fig. 2a). However, during the late winter to early spring of 2004, a high emigration rate, mortality from unknown reasons, and absence of nest and chicks of Black-necked swans were observed within this Ramsar site (13). In addition, a significant reduction in the spatial extent and even death of the aquatic, submerged macrophyte, locally known as Luchecillo (*Egeria densa*),

were also observed. This plant was the primary food source for the swans and other herbivorous birds, such as coots (*Fulica armillata* and others), both being the most abundant water birds in the site (nearly 90% of the total avian population). In November 2004, the Chilean National Environmental Commission (CONAMA) contracted Universidad Austral de Chile to carry out the study “On the origin of mortality and population decline in aquatic birds from the Carlos Anwandter Natural Sanctuary, Province of Valdivia.” The final report of this study was due in April 2005 (13). Since then, several other studies have been performed (see www.humedalriocruces.com). Here we report the main findings related to the emigration and death of swans and the disappearance of *E. densa*.

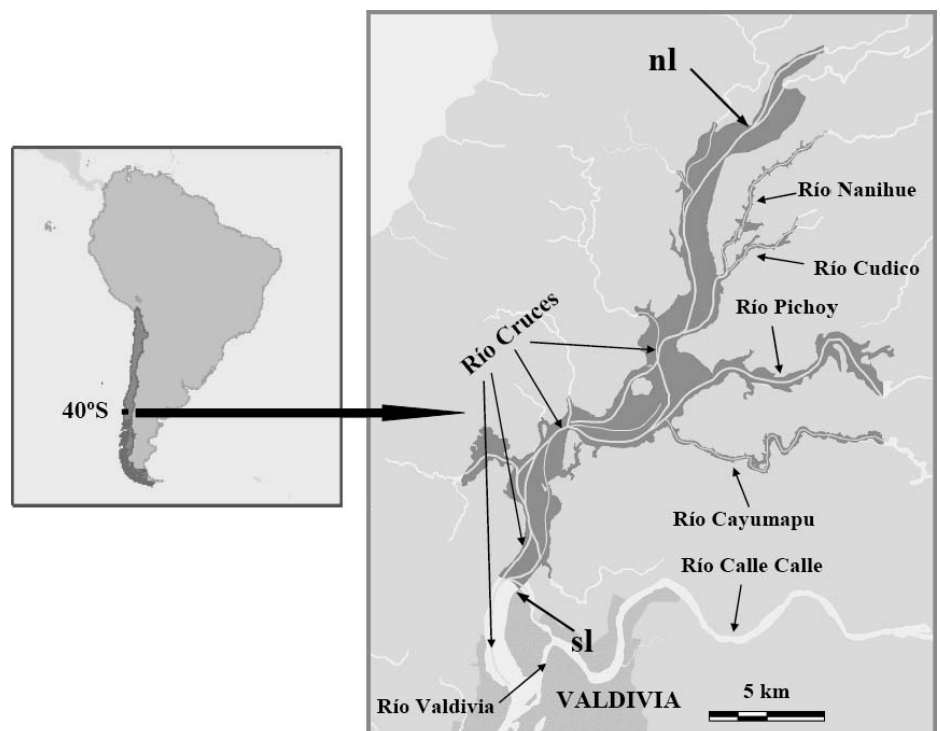


Figure 1. Geographic location of the wetland of Río Cruces or the sanctuary (darker areas around río Cruces), northward of the city of Valdivia, southern Chile. The darker areas are shallow water zones primarily represented by swamps and marsh vegetation that originated after the earthquake and tsunami of May 1960. Note the different branches of Río Cruces throughout all the shallow water areas. nl and sl = northern and southern limits of the sanctuary, respectively.

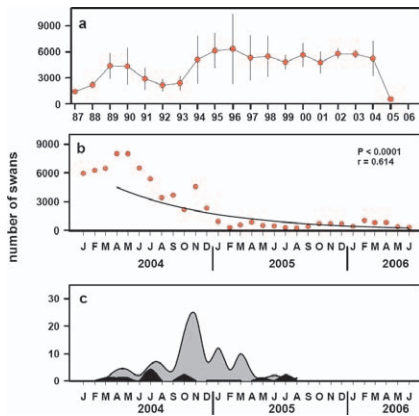


Figure 2. (a) Inter annual variability in population abundances of Black-necked swans at the sanctuary; the vales are means \pm one standard deviation. (b) Temporal variability in the monthly population abundance of swans from January 2004 to June 2006; regression equation calculated from April 2004 onward. (c) Monthly variability in number of swans found dead: grey area = unknown mortality, black = known mortality. Data source: CONAF (Corporación Nacional Forestal, Chile).

CHARACTERIZING BIOTIC CHANGES

The swan population began to drop significantly in April and May 2004, from nearly 8000 to less than 400 during May and June 2006 (Fig. 2b). Concurrently with this population drop, the number of swans found dead for unknown reasons increased from 4 (April–May 2004) to a maximum of 18 and 24 during October and November 2004, respectively; afterwards, that number decreased to 1 or 2 in May 2005 (Fig. 2c). On the other hand, the number of swans found dead for known reasons (e.g., gun shot, collision with electricity cables) was fairly constant throughout this period. Macroscopic analyses of swans found dead ($n = 20$) or moribund ($n = 22$) for unknown reasons within the sanctuary and nearby wetlands revealed a high degree of emaciation in most birds (Fig. 3a). Worth noting was the low frequency of plant material in the stomach content of those birds and their low weight (mean = 3.9 kg \pm 1.0 SD, $n = 42$), compared to the weight of swans sampled from the sanctuary studied during June to September 2003 (mean = 5.5 kg \pm 1.0 SD, $n = 52$) (14) (Fig. 3a and b).

Light microscopy analyses of swan livers collected within the sanctuary showed that all of them had granular dark brown pigments in hepatocytes, which stained blue with Prussian blue stain, an iron-specific stain. In contrast, a slight granular brown pigmentation was found in just one of four swans collected alive from Puerto Natales (ca. 51°S), nearly 1400 km south of the sanctuary and examined for comparative purposes. The presence of a high overload of iron in the hepatocytes of the swans of the sanctuary and nearby wetlands was confirmed by atomic absorption spectrometry flame analyses, showing that the iron content

of the livers (mean = 15 375.9 mg kg dry weight \pm 8360.5 SD) was significantly higher ($H = 7.25$, $P = 0.01$) than in the swans' livers collected from Puerto Natales (mean = 5737.0 mg kg dry weight \pm 815.8 SD) and whose hepatocytes were negative to Prussian blue stain. Results of microscopic and iron content analysis indicated that swans from the sanctuary and nearby wetlands were affected by high absorption of iron from the diet with respect to needs, resulting in liver histopathologic alterations, a condition commonly reported in captive birds (15–18).

Concurrent with the emigration and death of Black-necked swans, a widespread decrease in the cover of the macrophyte *E. densa* within the sanctuary and nearby tributary rivers was also observed. This aquatic macrophyte was the dominant species of the wetland plant community, extending in shallow waters down to 4 m deep all along the sanctuary (6, 7). At the present, the cover of *E. densa* is sparse and patchy; in contrast, healthy stands of plants are found along the borders of Río Calle Calle and Valdivia, nearly 5 km outside the sanctuary (Fig. 1). The stems and leaves of plant remains of *E. densa* collected within the sanctuary had a brown color, necrotic areas, and a crust composed of diatom species and salts, contrasting with the green color of healthy plants collected in those outside areas (Fig. 3c,d). That brown coloring is the result of coalesced necrotic tissue, which is the typical symptom of plants exposed to high levels of iron. This promotes an increase in the formation of reactive oxygen-based radicals, which damage vital cellular constituents, such as membranes, by lipid peroxidation (19–26). The iron content in plants collected within the sanctuary and nearby wetlands during December 2004 ($n = 13$ sites or samples) varied between 9553.0 and 48 338.0 mg kg dry weight, with a mean of 33 445.9 mg kg dry weight (SD = 10 016.7). That mean was nearly three times higher than the mean iron content obtained from two sites (samples) collected during that month outside the sanctuary (Río Calle Calle and Valdivia, 11 375.0 mg kg dry weight). Based on the above, we concluded that high concentrations of iron and other heavy metals (13) were the most probable cause for the massive mortality of *E. densa* in the sanctuary. Moreover, the crust precipitated over leaves and stems probably interfered with photosynthesis by blocking sunlight and also contributed to the demise of the underwater plant cover within this Ramsar site.

The earlier results led us to conclude that Black-necked swans emigrated from the sanctuary due to the demise of its main food, *E. densa*. Many of those not able to emigrate were affected by emaciation and high loads of iron in their livers, leading to death. These conclusions moved us to ask, “Why did iron precipitate over the plant leaves and stems consumed by the swans?”

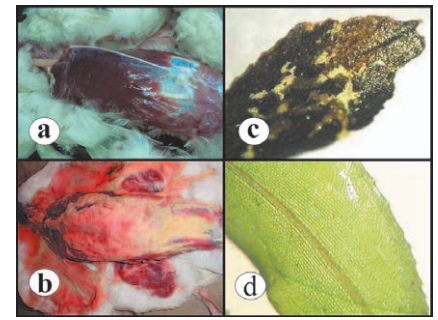


Figure 3. (a) A swan collected within the sanctuary in November 2004. Note emaciation with no fatty deposits on the chest (see sternum with the visible keel). (b) Picture of a swan collected in the same area as the former bird, but during September 2003. Note the abundant fatty deposits over the pectoral muscles and the visceral cavity body, as well as the subdermal fat on skin. (c) Surface leaves of *Egeria densa* collected within the sanctuary during December 2004, showing the necrotic brown areas, as opposed to the clean surface of plant leaves collected from río Calle Calle (d), nearly 5 km outside the sanctuary.

The final report of Universidad Austral de Chile concluded that high concentrations of iron and other heavy metals over the plants were related to significant changes in water quality that occurred spatially and temporarily in the Río Cruces (13), after a large new wood pulp mill (located nearly 25 km upstream of the sanctuary) began to operate during February 2004 to boost its exports of eucalyptus and monterrey pine pulp. Aluminum-based coagulant (i.e., aluminum sulfate) is used in the chemical treatment of the liquid wastes of the mill to coagulate particles. Because some of the water quality changes are increases in sulfate and aluminum downstream of the mill, it is assumed that an excess of aluminum in the sanctuary resulted in the precipitation of iron and other heavy metals on the aquatic plants (13). Because the extensive stands of *E. densa* contributed to sedimentation in the past, the present absence of them resulted in resuspension of sediments enriched with heavy metals (13) that also contributed to iron precipitation over the plants. Thus, changes in water quality of the Río Cruces and tributary rivers have been the main driver of the significant biotic changes that occurred in this Ramsar site since mid 2004.

THE FUTURE

The dramatic biotic changes occurring in the sanctuary as a result of water quality changes induced by the discharges of the waste water of the pulp mill into the river upstream this Ramsar site have promoted a discussion about the role of the Chilean environmental policies, which, under uncertain or eventual environmental impacts, authorized those discharges. During June 2005, the Regional Environmental Commission (COREMA) resolved that no later than 2007, the pulp mill will have to avoid

the use of Río Cruces as a recipient of its liquid effluents. One of the new pathways for those waste waters could be the ocean waters nearly 30 km west of the plant. However, the possible construction of a pipeline to the ocean provokes concern within the fishermen living in a wide coastal area and about the uncertainty of environmental effects similar to those reported for the wetland of Río Cruces. These would probably affect seaweed, shellfish, and fish, which represent the fishermen's main income. Thus, the sudden biotic changes occurring in the Ramsar site of Río Cruces highlight the need for a systematic application of the precautionary principle as a management tool (27) to be used by the Chilean environmental agencies, especially in areas where scientific information is still scarce. With the purpose of obtaining the basic and needed knowledge to evaluate the viability of mitigation and restoration strategies, scientists from Universidad Austral de Chile and other Chilean universities recently designed a cross-disciplinary research program regarding the study of the abiotic and biotic components at several spatiotemporal scales and across several biological organization levels. This is to implement an ecosystem management plan to monitor the pathways that this wetland will follow and the strategies needed to ensure its restoration. These universities are also concerned about the need to improve environmental policies regarding the use of wetland ecosystems, not only in southern Chile, but throughout the country.

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