Incompatibility of sulphate compounds and soluble bicarbonate salts in the Rio Cruces waters: an answer to the disappearance of *Egeria densa* and black-necked swans in a RAMSAR sanctuary

Sandor Mulsow¹,²,*  Mariano Grandjean¹

¹Instituto de Geociencias, and ²Forest Ecosystem Services Research (FORECOS), Universidad Austral de Chile, Casilla 567–Valdivia, Chile

ABSTRACT: The Carlos Anwandter Sanctuary, a RAMSAR site, is situated downstream from the junction of the Rio Cruces and Rio Calle–Calle near Valdivia in Southern Chile. The Rio Cruces was a bicarbonate-rich aquatic ecosystem until January 2004, when a pulp mill began pouring >40 t of sulphate (SO₄) and 6 to 9 t of H₂SO₄ each day into the River. Soon after, black-necked swans, which take refuge in the Sanctuary, began to die and emigrate. Previous studies showed that the food of the birds, the submerged vascular plant *Egeria densa*, had been eliminated from areas of the Sanctuary affected by the mill’s effluent. Here we describe the cause of this loss of plants and birds. *E. densa* is a C₄ plant that uses calcium bicarbonate to compensate for low concentrations of CO₂ in its local environment. Without calcium bicarbonate in the water, the plant photorespires, loses turgor, turns brown and dies. Here we demonstrate that the sulphate and acid dumped into the river by the pulp mill caused an anionic/cationic disequilibrium resulting in the precipitation of both Mg²⁺ and Ca²⁺ oxides — as brucite and portlandite — and other metallic (Fe, Al, Cu) oxides which enhanced the concentration of H⁺, thus perpetuating the ionic disequilibrium. Further, aqueous sulphate under light acidic conditions could precipitate gypsum, thus further removing calcium from the water. An ecologically and statistically significant loss of calcium bicarbonate (ANOVA, p < 0.05) is shown: samples of water affected by the mill’s effluent contained 37% less (HCO₃⁻) than those collected outside the area affected by the effluent. In a microcosm experiment, *E. densa* samples were exposed to no sulphate (4 replicates: control), 2.5 g l⁻¹ of K₂SO₄ (4 replicates: Treatment 1), 4.9 g l⁻¹ of K₂SO₄ (4 replicates: Treatment 2), and 9.8 g l⁻¹ of K₂SO₄ (4 replicates: Treatment 3). Plants in the control microcosms produced oxygen through photosynthesis at a rate of 0.24 ml O₂ g⁻¹ h⁻¹. Plants in Treatment 1 produced oxygen at half the rate of the controls: 0.11 ml O₂ g⁻¹ h⁻¹. Plants in Treatment 2 produced oxygen at a rate of 0.003 ml O₂ g⁻¹ h⁻¹ or 2 orders of magnitude lower than plants in the control microcosms. After only 8 h of exposure to the experimental conditions of Treatment 3, the plants produced oxygen at a rate of only 0.0001 ml O₂ g⁻¹ h⁻¹ or 3 orders of magnitude lower than controls. Differences in oxygen production among the controls and treatments were statistically significant (ANOVA, p < 0.05).

KEY WORDS: Pulp mill · C₄ · Photosynthesis · *Egeria densa* · Environmental disaster · CO₂ concentration mechanisms · CCM · Bicarbonate salts · Industrial pollution

INTRODUCTION

The Carlos Anwandter Sanctuary in Southern Chile is a RAMSAR site—an area of wetlands of international importance, designated as such under the United Nations Convention on Wetlands signed in Ramsar, Iran (UNESCO, 1971). The area is downstream from the junction of the Cruces and Rio Calle–Calle near Valdivia. The land formation was altered by the great 1960 earthquake and is now composed of slow moving streams, swamps and a tidal estuary that empties into the Pacific Ocean at the Bay and Port of Corral. The Sanctuary offers breeding grounds for a vast array of waterfowl. Eco-tourism is economically important to the area. The Sanctuary is now threatened by forestry plantations, agriculture, cattle husbandry, small and large urban waste water treatment facilities and, most recently, effluent from a
new pulp mill. More than 90% of the waste water released into the Río Cruces comes from the CELCO–ARAUCO pulp mill.

In January 2004, more than 6000 black-necked swans were counted in the Sanctuary. During April to May 2004, dozens of these swans were found dead, floating in the waters of the Sanctuary. After July, only a few hundred were left. By then, most of the black-necked swans had emigrated from the Sanctuary and more than 200 swans had been found dead. The local authorities ordered an ecological assessment study to establish the cause of the disappearance and death of the birds. The local university, Universidad Austral de Chile (UACH), undertook exhaustive studies to define and eliminate hypotheses to explain the death of the birds (UACH 2004, 2005). The principal outcome of these studies was that the main food source for the birds, a vascular sub-aquatic plant, Egeria densa, had been completely eliminated from the Sanctuary. However, the studies did not identify the cause of the loss of E. densa. Here we report an experiment designed to determine why the plant disappeared.


Submersed plants, algae and cyanobacteria among others, compensate for these adverse conditions through biophysical or proton extrusion mechanisms (together referred to as CO2 concentration mechanisms, CCM). These mechanisms convert soluble calcuumbicarbonate (HCO3)– to CO2. At least 3 species of Hydrocharitaceae — Hydrilla verticillata (Bowes et al. 1979, Holaday et al. 1983, Moore et al. 1987, Morton & Keeley, 1990, Magnin et al. 1997), Egeria densa (Dai et al. 1993, Casati et al. 2000) and Elodea canadensis (de Groote & Kennedy 1977) — show evidence of C4 metabolism associated with CCM (Bowes et al. 1971). Badger & Spalding (2000) proposed a generic model to describe CCM in submersed plants, such as E. densa: the poor affinity of RubisCo for CO2 and its relatively high affinity for O2 requires that the plants increase intracellular partial pressures of CO2 incorporating soluble salts of (HCO3)– present in the surrounding water, otherwise E. densa cannot photosynthesize (see Leegood 2002 for details). Calcium bicarbonate in water can be easily disassociated by a stronger anion such as SO4^2–. It is also affected by pH < 7.0 and by high temperatures.

In January 2006, CELCO–ARAUCO began operation of a kraft-bleached type pulp mill on the Río Cruces above the RAMSAR Sanctuary. The mill has an annual capacity limited to 550 000 t (WWF 2005). It discharges SO4 into the river at a rate of >40 t d^–1 (the pulp mill stated officially that the discharge of sulphate is in the order of 40 t d^–1, but they have been fined for operating in excess of this limit). Aluminum sulphate, similar to ALUM™, a hydrated salt of aluminum, coagulates suspended material (Al3+) and clarifies the effluent before it leaves the mill. The plant also reports the discharge of 6 to 9 t d^–1 of H2SO4.

Before the pulp mill began operations, (1) Campos (1995) characterized the Río Cruces as a freshwater river buffered with bicarbonate concentrations of 27.9 ± 8.7 (SD) mg (HCO3)– l–1 (n = 7) and (2) water at a sampling station (named Puente Rucaco) located 1 km downstream of the pulp mill had concentrations of SO4 < 0.6 ± 0.3 (SD) mg SO4 l–1 (n = 20) (DGA 1993). In February 2006 at the same station, we measured concentrations of SO4 as high as 4.5 mg l–1 — more than 7 times its historic levels.

**MATERIALS AND METHODS**

In February–March 2006, 2 yr after the pulp mill began production, we sampled 11 stations along the Río Cruces. Two stations were located upstream of the effluent discharge (Table 1), 1 station was 1 km downstream of the effluent discharge (Puente Rucaco, Table 1), 6 stations were located within the limit of the Sanctuary and 2 were located in the Río Calle–Calle. The samples were collected using a van Dorn bottle from a research vessel at the stations located in the Sanctuary and Rio Calle–Calle and by hand using SCUBA equipment at stations located upstream and downstream of the pulp mill.

Concentrations of CO2 and (HCO3)– were analyzed on site using titration techniques (Parson et al. 1985), and pH was measured using an Orion temperature corrected pH meter. Water samples for analysis of major cations and anions were collected using a high-density plastic bottle and kept cool before being sent to SERNAGEOMIN, an ISO 9000 credited Laboratory, for analysis. Cations and anions were determined using Ionic DX Chromatography. The cation/anion concentration values were then entered into WATEQ4F (Ball & Nordstrom 2001) to evaluate the cation/anion equilibrium.
For experiments, complete and healthy *Egeria densa* plants as well as water were collected from Stn Calle–Calle 2 (see Table 1); these samples were considered unaffected by the pulp mill effluent. Water and plants were transported immediately in a cooler to the Geobenthos Laboratory at UACH. Once in the Laboratory, four 100 l glass aquaria were filled with tap water and kept at 14 to 16°C with a custom made chilling device. In each aquarium, four 10 l glass microcosms were arranged, aerated and kept out of contact with the refrigerated water. In each of these, we placed a section of *E. densa* stem with verticillata in good condition and including the apex (0.6 to 0.8 g dry wt) inside an inverted, transparent gas-tight funnel (bio-oxygen meter). At the tip of the funnel, a plastic syringe was inserted and filled with water. Oxygen produced by the plant rose into the tip of the funnel displacing water. Oxygen production was used to estimate the photosynthesis rate of the plant (ml O₂ g⁻¹ h⁻¹).

To test the effects of sulphate effluent on the plants, all 10 l microcosms described above were fitted with a bio-oxygen meter and a sample of *Egeria densa* and allowed to produce oxygen for 8 h. In this way, the natural photosynthetic rate was determined for each plant. Then, 4 individual plant samples (replicates) were designated randomly to one of 4 control replicates or to one of 4 replicates of 3 different treatments. The 4 control replicates had no addition of K₂SO₄. The 4 replicates of Treatment 1 received a dose equivalent to 15 d of sulphate released by the pulp mill (specific concentration of 2.5 g SO₄ l⁻¹). The 4 replicates of Treatment 2 received the equivalent of 30 d of sulphate release (specific concentration of 4.9 g SO₄ l⁻¹) and the 4 replicates of Treatment 3 received the equivalent of 60 d of sulphate effluent (specific concentration of 9.8 g SO₄ l⁻¹).

Calculation of the equivalent doses was based on the company’s own estimate of 40 t d⁻¹ of sulphate release by the pulp mill—a estimate lower than that provided by UACH (2005) which, in December 2004, stated that CELCO–ARAUCO discharged 59 t d⁻¹ of sulphate salts. We then used a dilution factor for the Sanctuary of 250 × 10⁶ l to arrive at 15 d (2.5 g SO₄ l⁻¹), 30 d (4.5 g SO₄ l⁻¹) and 60 d (9.8 g SO₄ l⁻¹) equivalent doses of sulphate in the treatment microcosms. Statistitical analyses were carried out using a PC program Statistica™. Differences were considered significant at p < 0.05.

### RESULTS

#### Fieldwork

The concentration of (HCO₃)⁻ was statistically significantly higher at stations located upstream and downstream of the pulp mill effluent than that at stations located within the Sanctuary boundaries downstream of the pulp mill effluent, including Puente Rucaco (1-way ANOVA, p < 0.005). In general, the samples outside the affected parts of the Sanctuary had up to 37% more (HCO₃)⁻ than those collected inside the area affected by the pulp mill effluent (Table 1). The concentration of SO₄ was 3 times higher at Rucaco and at stations within the Sanctuary than that at stations located outside the areas influenced by the pulp mill; these differences are ecologically and statistically significant (1-way ANOVA, p < 0.05) (Table 1).

The concentration of SO₄ was 3 times higher at Rucaco and at stations within the Sanctuary than that at stations located outside the areas influenced by the pulp mill; these differences are ecologically and statistically significant (1-way ANOVA, p < 0.05) (Table 1).

There was a strong negative correlation (r² = 0.81; p < 0.05) between the concentration of SO₄ and HCO₃; thus, the higher the concentration of sulphate, the lower the

### Table 1. Bicarbonate concentrations measured in the water column. Values are means ± SD of 6 replicates from 11 stations located upstream and downstream of the pulp mill effluent. Shaded rows represent stations located within the limits of the Carlos Anwandter Sanctuary that have been heavily impacted by the effluent.

<table>
<thead>
<tr>
<th>Stn</th>
<th>Latitude (°S)</th>
<th>Longitude (°W)</th>
<th>Depth (m)</th>
<th>SO₄ (mg l⁻¹)</th>
<th>Alkalinity (mg HCO₃ l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puente Cruces</td>
<td>39° 26' 9.9</td>
<td>72° 45' 35.9</td>
<td>1</td>
<td>0.70</td>
<td>29.48 ± 1.76</td>
</tr>
<tr>
<td>Puente Negro</td>
<td>39° 29' 21.6</td>
<td>72° 48' 33.8</td>
<td>1</td>
<td>0.77</td>
<td>35.07 ± 10.78</td>
</tr>
<tr>
<td>Puente Rucaco</td>
<td>39° 33' 6.6</td>
<td>72° 54' 8.0</td>
<td>1</td>
<td>4.52</td>
<td>24.40 ± 3.05</td>
</tr>
<tr>
<td>Isla Teja</td>
<td>39° 47' 15.0</td>
<td>73° 16' 31.3</td>
<td>0</td>
<td>3.37</td>
<td>20.33 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>3.39</td>
<td>28.46 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>3.40</td>
<td>17.28 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>3.23</td>
<td>14.23 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>2.82</td>
<td>14.23 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>3.12</td>
<td>19.31 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td>3.17</td>
<td>16.26 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>2.58</td>
<td>21.35 ± 3.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>2.58</td>
<td>21.35 ± 3.00</td>
</tr>
<tr>
<td>Puente Rucaco</td>
<td>39° 47' 5.1</td>
<td>73° 15' 53.0</td>
<td>0</td>
<td>1.88</td>
<td>21.35 ± 3.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>3.39</td>
<td>28.46 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>3.40</td>
<td>17.28 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>3.23</td>
<td>14.23 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>2.82</td>
<td>14.23 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>3.12</td>
<td>19.31 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td>3.17</td>
<td>16.26 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>2.58</td>
<td>21.35 ± 3.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>2.58</td>
<td>21.35 ± 3.00</td>
</tr>
<tr>
<td>Calle–Calle 1</td>
<td>39° 48' 53</td>
<td>73° 14' 1.5</td>
<td>0</td>
<td>1.37</td>
<td>26.43 ± 1.76</td>
</tr>
<tr>
<td>Calle–Calle 2</td>
<td>39° 49' 12.4</td>
<td>73° 13' 23.6</td>
<td>0</td>
<td>1.26</td>
<td>29.48 ± 1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>3.34</td>
<td>32.53 ± 4.65</td>
</tr>
</tbody>
</table>

*Puente Rucaco station is located 1 to 2 km downstream of effluent discharge and has the highest value of sulphate recorded and reported here.

bAt this station water and plants were collected for the experimental work.
concentration of HCO$_3^-$.

Furthermore, in the period 1987 to 1992, Puente Rucaco station had SO$_4^-$ concentrations 5 times lower than those observed today (0.6 ± 0.3 mg SO$_4^-$ l$^{-1}$, n = 20; Rio Rucaco: 1987 to 1992; DGA 1993).

All pH, temperature and cation and anion concentrations (HCO$_3^-$, SO$_4^{2-}$, Cl$^-$, NO$_3^-$, Ca$^{2+}$, Mg$^{2+}$, Na$^+$ and K$^+$) measured for the 11 sample stations were analyzed using WATEQ4F (Ball & Nordstrom, 2001) to examine the tendency of water to reach mineral solubility equilibria as a constraint on interpreting the chemistry of natural waters (Ball & Norstrom, 2001). The calculated differences in cation/anion balance between stations affected by the pulp mill effluent and those unaffected were ecologically and statistically significant (ANOVA, p < 0.05). When we compared our data with the historical data collected by the Dirección General de Aguas de Chile (DGA) during 1987 to 1992, sites unaffected by the effluent today were not statistically different from the historical data, while sites downstream from the pulp mill effluent today were.

The chemical changes outlined above coupled with rises in temperature and decreases in pH has induced the formation of fulvic–humic complexes—yellow substance or Gelbstoff—that now colors and clouds the waters of the Sanctuary. The presence of anions increased conductivity dramatically to levels higher than 6000 µS cm$^{-2}$ (S. Mulsow pers. obs.).

Egeria densa was only found in waters with [HCO$_3^-$] concentrations greater than 26 mg (HCO$_3^-$) l$^{-1}$ and geographically was only found in the waters of the Rio Calle–Calle that were unaffected by the pulp mill effluent. E. densa was not found when the water concentration of [HCO$_3^-$] was less than 20 mg (HCO$_3^-$) l$^{-1}$ or SO$_4^{2-}$ concentration was higher than 1.8 mg SO$_4^{2-}$ l$^{-1}$. SO$_4^{2-}$ was the only major chemical compound that was not found when the water concentrations decreased by orders of magnitudes compared to controls, and the differences were statistically significant (Fig. 2). Controls produced oxygen at a rate of 0.24 ml O$_2$ g$^{-1}$ h$^{-1}$. Plants in Treatment 1 (2.5 g SO$_4^-$ l$^{-1}$) produced oxygen at half the rate of the controls: 0.11 ml O$_2$ g$^{-1}$ h$^{-1}$. Plants in Treatment 2 (4.9 g SO$_4^-$ l$^{-1}$) and 60 d (9.8 g SO$_4^-$ l$^{-1}$), photosynthetic rates decreased by orders of magnitudes compared to controls, and the differences were statistically significant (Fig. 2).

Experimental work

The rates of photosynthetic production of oxygen in the initial experiment in all microcosms—those used as controls or treatment vessels—were not statistically different (Fig. 1, no addition of sulphate; ANCOVA, p < 0.05). However when the same plants, were exposed to a dose of SO$_4^-$ salt equivalent to 15 d (2.5 g SO$_4^-$ l$^{-1}$), 30 d (4.9 g SO$_4^-$ l$^{-1}$) and 60 d (9.8 g SO$_4^-$ l$^{-1}$), photosynthetic rates decreased by orders of magnitudes compared to controls, and the differences were statistically significant (Fig. 2). Controls produced oxygen at a rate of 0.24 ml O$_2$ g$^{-1}$ h$^{-1}$. Plants in Treatment 1 (2.5 g SO$_4^-$ l$^{-1}$) produced oxygen at half the rate of the controls: 0.11 ml O$_2$ g$^{-1}$ h$^{-1}$. Plants in Treatment 2 (4.9 g SO$_4^-$ l$^{-1}$) produced oxygen at a rate 2 orders of magnitude lower than plants in the control microcosm: 0.003 ml O$_2$ g$^{-1}$ h$^{-1}$. After only 8 h of exposure to the experimental conditions of Treatment 3 (9.8 g SO$_4^-$ l$^{-1}$), the plants produced oxygen at a rate 3 orders of magnitude lower than those in the control microcosm: 0.0001 ml O$_2$ g$^{-1}$ h$^{-1}$.

The only compound added to the treatment microcosms was SO$_4^-$ in the form of K$_2$SO$_4$ (Merck grade). The initial conditions (pH, CO$_2$ concentration, HCO$_3^-$) of the water in the controls did not change during the experiment. However, these variables changed in the treatment microcosms. A change in pH of 0.4 over the course of the experiment (Table 2) that indicates an ecologically significant decrease in the concentration of (HCO$_3^-$) had occurred. In all the treatments, (HCO$_3^-$) decreased 10 to 30% compared to initial concentrations and, in these microcosms, Egeria densa decreased its photosynthetic rate by orders of magnitude (Table 2). The plants lost rigidity and turned brown; their health deteriorated.
DISCUSSION

We have demonstrated that the addition of SO$_4$ decreased the concentration of (HCO$_3$)$^-$ in the experimental microcosms. The concentration of CO$_2$ was similar before and after the experiment. *Egeria densa*, a C$_4$ vascular plant that uses (HCO$_3$)$^-$ salt uptake as a CO$_2$ concentrating mechanism could not photosynthesize (generate oxygen) when exposed to low concentrations of SO$_4$.

A similar phenomenon must have occurred in the Sanctuary after the CELCO–ARAUCO pulp mill began operations in January–February 2004 (Table 1). As a consequence of the mill's discharge of SO$_4$, concentrations of this toxin rose 5 to 6 times in the Rio Cruces and 3 to 4 times in the waters of the Sanctuary downstream. Calcium bicarbonate decreased 1.6 times in the same waters. When a similar decrease occurred in our experiment, the plants responded by reducing photosynthesis and they lost their rigidity and turned brown. Their health deteriorated.

*Egeria densa* is a C$_4$ vascular submersed plant that uses calcium bicarbonate to concentrate CO$_2$ near its RuBisCo enzyme to enhance the enzyme’s photosynthetic capacity in conditions of low CO$_2$ and high O$_2$ concentrations (Bowes et al. 1971, 1979, Leegood et al. 2002). When the plant is confronted with low calcium bicarbonate concentrations and resulting low CO$_2$, and high O$_2$, RuBisCo is unable to catalyze photosynthesis and begins to catalyze photorespiration instead.

All previous studies of the ecological disaster in the Carlos Anwandter Sanctuary following the onset of operations at the CELCO–ARAUCO pulp mill were observational and presented correlations only, not cause and effect relationships (UACH 2004, 2005). These studies observed that only submersed vascular plants died off after the mill opened and only in areas affected by the mill’s effluent. The plants lost turgor, displayed deposits on their surfaces, turned brown and died (UACH 2004, 2005). The submerged vascular plant *Egeria densa* is the major source of food for the black-necked swans that take refuge in the Sanctuary. When this plant died as a result of the SO$_4$ released by the mill, as demonstrated here, the birds died or emigrated from the Sanctuary. The birds, like the plants in the treatment microcosms, showed signs of low resilience to environmental stresses that they had previously survived, displayed enhanced colonization by parasites and exhibited low rates of depuration of metals (UACH 2004, 2005). The health of the birds

<table>
<thead>
<tr>
<th>pH</th>
<th>Temp.</th>
<th>CO$_2$</th>
<th>HCO$_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.8</td>
<td>15.0</td>
<td>1.95 ± 0.47</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>7.9</td>
<td>14.5</td>
<td>1.47 ± 0.25</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>7.8</td>
<td>14.5</td>
<td>1.76 ± 0.44</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>7.7</td>
<td>15.0</td>
<td>1.70 ± 0.54</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH</th>
<th>Temp.</th>
<th>CO$_2$</th>
<th>HCO$_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.7</td>
<td>15.0</td>
<td>1.87 ± 0.46</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>7.4</td>
<td>15.5</td>
<td>1.76 ± 0.44</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>7.3</td>
<td>16.0</td>
<td>1.46 ± 0.25</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>7.3</td>
<td>14.8</td>
<td>1.65 ± 0.61</td>
</tr>
</tbody>
</table>

Table 2. Microcosm experimental conditions for *Egeria densa* before and after addition of K$_2$SO$_4$. Water and plants were collected at Stn Calle–Calle 1, outside the affected Sanctuary.
deteriorated, just as the health of the plants had deteriorated in the treatment microcosms.

The present study demonstrates that SO$_4$ salts released by the CELCO–ARAUCO pulp mill forced the CO$_2$/HCO$_3$/CO$_3$ equilibrium towards an ecologically and statistically significant decrease in (HCO$_3^-$), a compound essential to _Egeria densa_'s photosynthetic production, health and survival.

We have reproduced the significant inverse relation of SO$_4$ concentration and (HCO$_3^-$) found in the 11 stations sampled. The presence of strong acids in the aquatic system disrupted the following equilibrium:

$$2[Ca^{2+}] + 2[Mg^{2+}] + [Na^+] + [K^+] + [H^+] \Leftrightarrow 2[CO_3^{2-}] + [HCO_3^-] + [Cl^-] + 2[SO_4^{2-}] + [OH^-] + [NO_3^-]$$

After losing the ionic equilibrium, addition of SO$_4^{2-}$ resulted in the loss of (HCO$_3^-$) according to:

$$H_2SO_4 + Ca(HCO_3) \Leftrightarrow CaSO_4 + 2CO_2 + H_2O$$

Thus, a strong negative correlation results between SO$_4$ and (HCO$_3^-$) as demonstrated here. At near neutral pH, this relationship can be summarized as:

$$(HCO_3^-) \Leftrightarrow 2[Ca^{2+} + Mg^{2+}] - 2[SO_4^{2-}]$$

The fact that the waters affected by the pulp mill effluent are supersaturated with calcium and magnesium oxides confirms the argument that SO$_4^{2-}$ has been the main cause of the ionic disequilibrium that resulted in the loss of the calcium bicarbonate in the water and the loss of the source of CO$_2$ needed by _Egeria densa_ to photosynthesis.

The chemical equations above can also be affected by increases in temperature and slight decreases in pH. In both cases, since CO$_2$ is at equilibrium with H$_2$CO$_3$, escape of CO$_2$ results in decreased concentrations of (HCO$_3^-$) because bicarbonate is a semi-soluble weak salt in natural waters.

It is precisely this partially dissolved salt of calcium bicarbonate that _Egeria densa_ needs for photosynthesis (Prins et al. 1982, Salvucci & Bowes 1983, Sand-Jensen et al. 1985, Gordon 1986) allowing the plants to grow and multiply, thus providing food to sustain the populations of black-necked swans. This calcium bicarbonate has been removed by addition of sulphate, seasonally elevated temperatures and slight acidification of the water.

**Acknowledgements.** The authors thank the students of Geobenthos Laboratory for their constant help while sampling and during experimental work. Partial funding from FONDECYT (grant no. 1050247) to S.M. and analysis and logistics from FORECOS are acknowledged. To Cedric and Fabrice, Sandor Mulsow’s sons, for their pristine environmetal concern and constant reminders throughout: What are you doing for the Sanctuary, Dad?

**Disclosure: conflicts of interest.** The authors have no personal, financial, political and/or other conflict of interest with the pulp mill or any other industries linked to the study area. The sole motivation of this research was to understand why an ecologically valuable submerged plant disappeared from the waters of the River Cruces and the Carlos Anwandter Sanctuary, a Ramsar site.

**LITERATURE CITED**


DGA (Direccion General de Aguas de Chile) (1993) Diagnóstico y Clasificacion de los cursos y cuerpos de agua segun objetivo de calidad: Cuenca Rio Valdivia. DGA, Ministerio de Obras Publicas, Santiago


Gordon DM (1986) Variable (HCO$_3^-$) affinity of *Elodea canadensis* Michaux in response to different (HCO$_3^-$) and CO$_2$ concentrations during growth. Oecologia 70:426–432


Long SP (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO$_2$ concentrations — has its importance been underestimated? Plant Cell Environ 14:729–739


Reiskind JB, Madsen TV, Van Ginkel LC, Bowes G (1997) Evidence that inducible C$_4$-type photosynthesis is a chloroplastic CO$_2$-concentrating mechanism in *Hydrilla*, a submersed monocot. Plant Cell Environ 20:211–220


UACH (Universidad Austral de Chile) (2004) Primer Informe sobre 'Estudio sobre origen de mortalidades y disminucion de aves acuaticas en el santuario de la naturaleza Carlos Anwandter, en la Provincia de Valdivia'

UACH (Universidad Austral de Chile) (2005) Segundo Informe sobre 'Estudio sobre origen de mortalidades y disminucion de aves acuaticas en el santuario de la naturaleza Carlos Anwandter, en la Provincia de Valdivia'


Submitted: April 20, 2006; Accepted: May 2, 2006

Proofs received from author(s): June 15, 2006