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DO SUBTOXIC LEVELS OF CHLORATE INFLUENCE THE DESICCATION TOLERANCE OF *EGERIA DENSA*?

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Abstract—Among the different factors hypothesized to be responsible for the virtual disappearance of *Egeria densa*, once a dominant aquatic macrophyte in a southern Chile wetland ecosystem, are the negative effects of certain chemical compounds (mainly chlorate) and harsh environmental conditions (desiccation caused by prolonged atmospheric exposure). The authors performed an integrated experiment in which *E. densa* plants were first exposed for four weeks inside a mesocosm system to levels of chlorate that existed in the wetland at the time of the plant's demise and then exposed to desiccation conditions that also resembled those that the system had experienced. Hence, the authors tested the hypothesis that *E. densa* plants exposed to sublethal levels of chlorate are more susceptible to the deleterious effect of desiccation compared with plants that had not been exposed to chlorate. This hypothesis was tested by means of quantifying physiologically related parameters in plants right after the four weeks under water and then after the desiccation period of 6 h. Their results rejected this hypothesis, because all plants, regardless of their history, are equally affected by desiccation. Environ. Toxicol. Chem. 2013;32:417–422. © 2012 SETAC

Keywords—Mesocosm Chlorate Aquatic macrophyte Desiccation

INTRODUCTION

Like many industrial liquid effluents, those produced by pulp mill plants contain a variety of compounds that must comply with environmental standards. Among those is chlorate (ClO_3^-), present in the discharge of elemental chlorine-free plants as a result of the application of chlorine dioxide (ClO_2), which is used in the cellulose bleaching process [1,2]. Chlorate is chemically stable in aquatic environments ([3]; E. Couture, 1998, Master's thesis, Dalhousie University, Halifax, NS, Canada), is highly soluble, and is not absorbed by particles in sediments [4]. In aquatic systems, the only significant toxicity attributed to chlorate has been demonstrated in the Baltic Sea in the growth of the brown algae *Fucus vesiculosus* and *Fucus serratus* exposed to low concentrations of this pollutant. Of these two species, *F. vesiculosus* is the more sensitive, with a no observed effect concentration value of 0.005 mg/L [5]. Furthermore, chlorate is unlikely to persist long in these systems because specialized populations of facultative bacteria are known to rapidly reduce chlorate to chloride in anaerobic sediments [3,6,7]. However, as has been postulated for other contaminants within the topic of ecological synergies [8], possibly sublethal concentrations of chlorate could interact synergistically with other pollutants or harsh environmental conditions to produce negative impacts on some ecosystems or their components [9].

In 2004, a wetland in southern Chile experienced an important ecosystem-level change when one of its main macrophytes *Egeria densa* virtually disappeared from the system [10]. Its trophic status as one of the main food items in the diet of the black-necked swan (*Cygnus melancoryphus*) prompted immediate public and scientific attention soon after swans started emigrating from the wetland [11]. Among the different hypotheses that were raised to explain the disappearance of *E. densa* was the supposedly toxic effect of chlorate. After more recent evidence provided by Marín et al. [12], who instead suggested that mass mortality of *E. densa* in the wetland during 2004 was attributable to the occurrence of an unusually dry and cold climatic event, a new chlorate-related hypothesis arose implying that dryness (i.e., plants exposed to air) was deleterious to plants already weakened by their exposure to sublethal levels of chlorate. Marín et al. [12] provided evidence that late fall 2004 was anomalous because of the presence of an atmospheric high-pressure cell that persisted most of the month of May over Southern Chile. This climatic event caused an almost complete absence of precipitation and low temperature conditions during this period. In addition, these authors performed ecophysiological experiments showing that 6-h exposure to desiccation kills this macrophyte. Similar desiccation conditions were considered by Bini and Thomaz [13] when proposing desiccation as the main explanation for the disappearance of *E. densa* and *Egeria najas* from the Itaipú reservoir in Brazil.

After considering these sources of evidence and with the main objective of testing the latter hypothesis (i.e., negative impact of desiccation), we quantified several different and relevant parameters in *E. densa* plants that were kept during one month in waters with elevated, yet realistic, levels of chlorate (similar to those they would have been exposed to in the wetland during early 2004) and were then exposed to plausible desiccation conditions (similar to those described to have occurred in 2004) for a period of 6 h (semidiurnal tidal period).

The general approach in the current study involved testing hypothesized sublethal effects of chlorate by recreating exposure conditions to chlorate using a mesocosm setting before plants were desiccated and physiologically related parameters were quantified in treatment and control plants.

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MATERIALS AND METHODS

Plants of *E. densa* were kept for four weeks inside 10 1,000-L experimental tanks that received a continuous flow (2 L/min) of water from the Cruces River. The general experimental setup was similar to that used in another related study [14]. One-half of the tanks (treatment tanks) received an additional continuous amount of chlorate via a mechanical dispenser (Dosatron) inserted in the line that fed them. This additional chlorate was delivered at a rate that would allow achieving a specific concentration inside the treatment tanks. During this period, treatment plants were exposed to two doses of chlorate: 0.7 and 1.4 mg/L for the first two and last two weeks, respectively (see justification for those concentrations later). Before being used in the experiment, a large number of plants collected in the nearby Calle-Calle River were acclimated for two weeks inside tanks with Cruces river water flowing through. Following a previously used methodology [14,15], only the top 30 cm (initial length) of the healthiest-looking plants were selected and considered for the experiment. Ten individually labeled plants were included inside each tank. The top 10 cm of each plant was marked with a fine tape to quantify apical growth (the increment in length from this mark). After four weeks, the following parameters were measured for each plant to detect any differences associated with the addition of chlorate: apical length (difference between final and initial length), number of apical leaves (leaves after the last node), number of apical nodes (those present between the tape and the tip of each plant), and number of newly formed roots and lateral ramifications.

During the desiccation phase (6 h, which simulates the exposure at extreme low water resulting from a combination of low river flow and low tide), having spent four weeks submerged in the mesocosm tanks, plants were kept at temperature and humidity levels resembling the average extremes recorded in the wetland during April and May 2004 [12]. Thus, plants were desiccated at 5 and 20°C (Dirección Meteorológica de Chile [2005], Climatic annual record [2004], Available at http://www.meteochile.gob.cl/Anuario/Anuario-2004.pdf). The 60% humidity considered also corresponds to the average value measured at the nearby Pichoy airport during April and May of 2004.

In addition to the morphometric variables mentioned above, after the plants spent four weeks submerged in the mesocosm tanks and 6 h of desiccation, the chlorophyll-a content of apical leaves and the photosynthetic metabolism (oxygen production and chlorophyll fluorescence) was quantified in 20 plants, 2 from each mesocosm. For this, 100 mg apical leaves (quick towel-dried) were ground with 25 ml 90% acetone until a green solution was obtained. This solution was vacuum-filtered, using Whatman GF/F 47 mm glass fiber filters. The absorbance was measured with a spectrophotometer (Hach DR 2700), following the procedure designed for samples extracted with acetone [16] and using the formulas by Lichtenthaler and Wellburn [17].

Before and immediately after desiccation, apical leaves of two additional plants from each tank were randomly selected and used for quantifying their photosynthetic metabolic performance (through the measurement of oxygen production rate) and chlorophyll-a fluorescence. For the oxygen rate quantification, we employed two metabolic chambers (3-ml capacity each) equipped with electrodes that were connected to a Strathkelvin 782 oxygen meter and were placed on top of magnetic stirring plates 20 cm apart. Approximately 0.025 g apical leaves were added to each chamber. The whole system was kept at 20°C with a thermoregulated bath (Lab Companion RW-1040G) that pumped water at a continuous rate of 3 to 5 L/min. Both chambers were illuminated with a metallic sodium halide lamp of 4,500°K (1,000 μE), positioned 15 cm away from both metabolic chambers. After 5 minutes of light exposure (enough time for stabilization of dissolved oxygen amount), the dissolved oxygen content inside each chamber was recorded. To standardize the oxygen quantifications among different plants after desiccation, a correction of the weight was necessary because of the water content of the plants. To determine the average humidity of wet plants, 0.5 g (quick paper towel–dried) leaves obtained from a subset of plants were weighed using a Sartorius BP-210S scale with a precision of ± 0.0001 g. Leaves were then oven-dried at 60°C until a constant weight was reached (for at least 120 min). This allowed us to obtain the average value (correction factor) for water content in leaves of *E. densa* of 11%, which was used when calculating the photosynthetic rate.

The chlorophyll-a fluorescence was quantified with a modulated fluorimeter (Hansatech Instruments, model FMS 1) to obtain maximal quantum efficiency of photosystem II F_v/F_m (where F_m is maximum fluorescence and F_v is variable fluorescence) in dark-adapted leaves, which is a commonly employed indicator of stress that quantifies the maximum quantum efficiency of the photosystem II, also referred to as the maximum photochemical primary efficiency of the leaves [18]. This parameter, typically in the range of 0.75 to 0.85 in healthy plants, is a sensible indicator of photosynthetic performance in plants [19]. The combination of chlorophyll fluorescence measurements together with net gas exchange parameters provides a good way to evaluate the photosynthetic performance in stressed plants [20] and to gain insight into the behavior of the photosynthetic machinery under stress [19]. For this quantification, apical leaves of two plants per tank were obtained and acclimated in the dark for 30 min inside Petri dishes filled with water from their original tanks. After this, each leaf was blot dried and the fluorescence measured, and the data immediately recorded in a portable computer.

Justification and criteria for the chlorate concentrations

The chlorate concentrations to which plants were exposed during the four-week experimental phase were chosen on the basis of simulations with a numerical hydrodynamic model of the only significant chlorate pulse from the Cruces River into the wetland during March to May 2004 [21]. A map of the wetland (Fig. 1) shows the areas with the maximum concentrations of chlorate simulated by the model and depicts the significant dilution of the chlorate pulse when it enters the wetland (point P1). The model also shows that most of the maximum chlorate concentrations fall into the 0 to 2 mg/L range, except for a reduced extension in the northern zone, where maximum concentrations were in the 2 to 4 mg/L range.

The chlorate level of 1.4 mg/L was chosen as the exposure concentration in the experiment because 32% of the wetland had this or higher concentrations, based on the modeling and March 2004 satellite images.

Figure 2 shows the temporal evolution of the chlorate concentrations in the Cruces River system at the point where it enters the wetland and in three other consecutive points (P1–P3 in Fig. 1). Because the concentration of 1.4 mg/L approximately corresponds to the maximum concentration at P2 (see Fig. 1), we decided to use a concentration profile similar to that at P2 (orange line in Fig. 2) in our experiment, including two weeks of exposure to 0.7 mg/L followed by two weeks at 1.4 mg/L of chlorate.

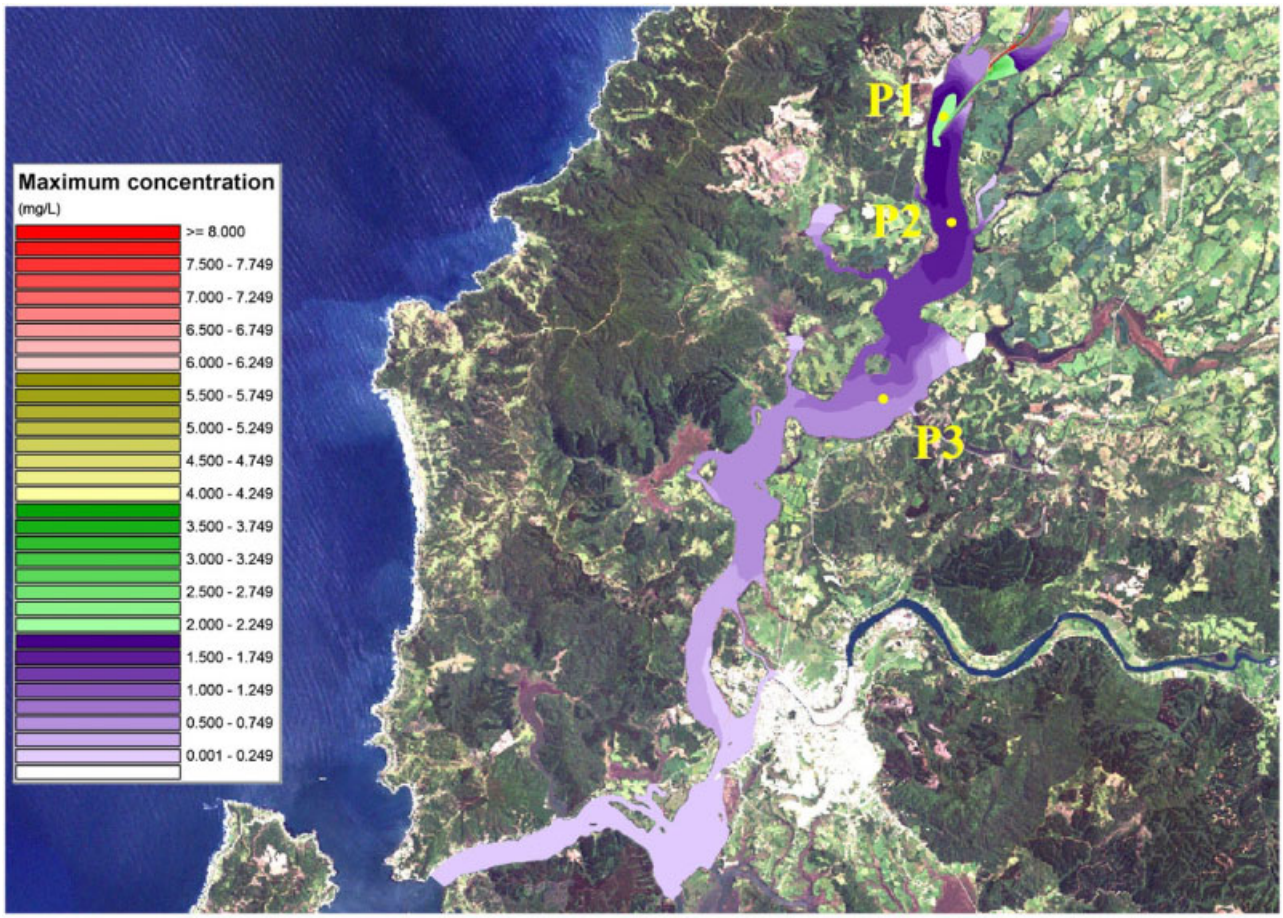

Fig. 1. Cruces River wetland showing the maximum chlorate concentrations simulated by a fate and transport model [20] between January 20, 2004, and May 30, 2004. Control points (P1–P3) start at the beginning of the wetland and extend to its mid-section.

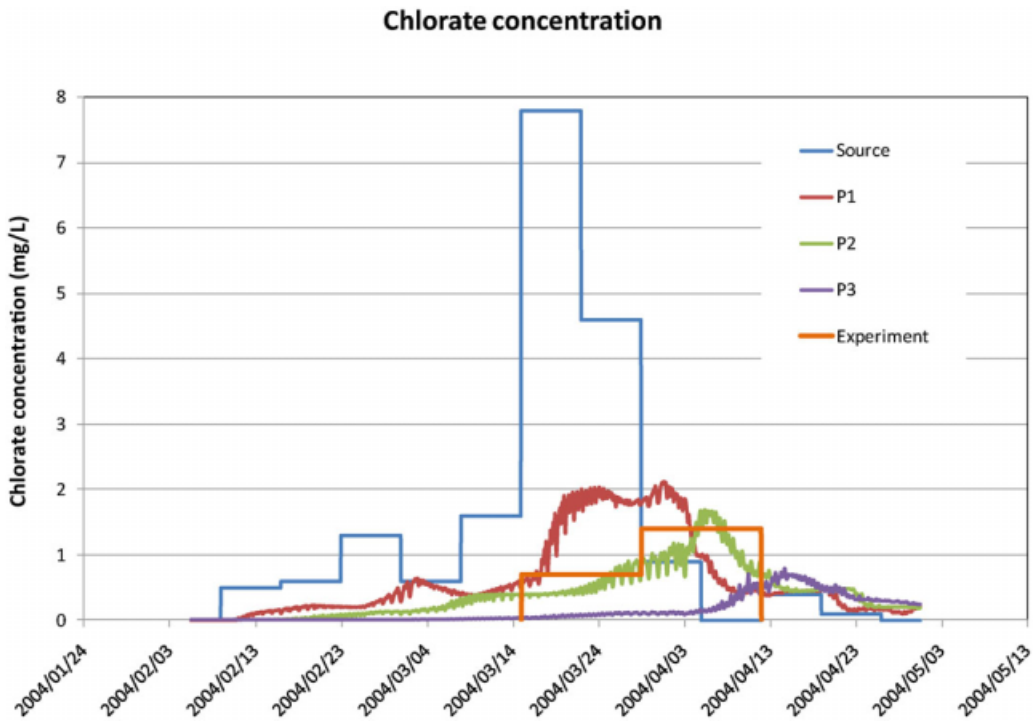

Fig. 2. Temporal evolution of chlorate concentration in the control points P1, P2, and P3 of the wetland. The blue line corresponds to the concentration of chlorate in the Cruces River when it enters the wetland. The orange line corresponds to proposed chlorate concentration profile used for four weeks in the present study.

Table 1. Average values of different parameters measured in plants after four weeks in flowing water with and without chlorate[a]

	Chlorate	Control	t_8	p
Apical growth (cm)	2.52 (1.99)	0.97 (0.77)	1.626	0.143
Number of apical nodes	23.59 (3.9)	19.8 (1.35)	2.053	0.074
Number of apical leaves	73.25 (14.76)	84.07 (10.47)	1.337	0.218
Number of ramifications	1.36 (0.65)	0.9 (0.22)	1.514	0.169
Chlorophyll-a (mg/ml)	6.57 (0.58)	6.32 (0.23)	0.911	0.389
Photosynthetic rate (mg O_2/h/g wet wt)	0.085 (0.076)	0.041 (0.082)	0.884	0.402
Chlorophyll fluorescence (F_v/F_m)	0.52 (0.04)	0.50 (0.04)	0.989	0.352

[a] Values in parentheses correspond to standard deviations. *T*-test values and probabilities are also provided.

RESULTS

Response of E. densa *to chlorate after four weeks*

None of the morphometric or physiologically related variables measured in plants that received 1.4 mg/L chlorate for four weeks (treatment) exhibited significant differences when compared with those that received only river water (Table 1).

Response of E. densa *to desiccation (photosynthetic rates)*

After two different desiccation conditions (6 h at 60% humidity/20°C and 6 h at 60% humidity/5°C), the photosynthetic rates of plants that had been exposed to chlorate for one month and that of control plants did not exhibit significant differences ($t_8 = 0.879$, $p = 0.405$, and $t_8 = 0.197$, $p = 0.848$; Fig. 3). These results show that only desiccation had a significant negative impact on the photosynthetic rate of *E. densa* plants (Table 2), even with a negative photosynthetic oxygen production (i.e., more respiration/decomposition than photosynthetic oxygen production), regardless of the air temperature to which plants were exposed. Moreover, all plants (those that had been exposed to chlorate levels for four weeks as well as the control plants) exhibited similar negative oxygen production after desiccation (Fig. 3, Table 2).

Response of E. densa *to desiccation (chlorophyll fluorescence)*

All plants examined reacted to the light stimulation, producing fluorescence. Higher values of F_v/F_m represent plants that allocated more energy to the photochemical process of photosynthesis. After four weeks, both plants exposed to chlorate and controls exhibited similar average fluorescence values ($t = 0.9888$, degrees of freedom = 8, $p = 0.3517$, Table 1). However, all fluorescence values were significantly lower after the 6-h desiccation period (Fig. 4, Table 3).

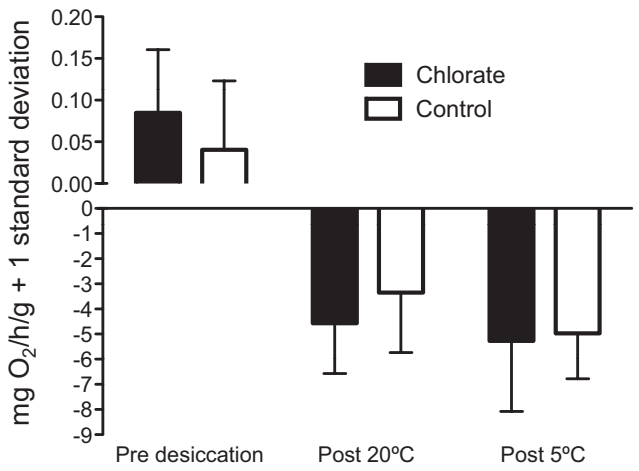

Fig. 3. Photosynthetic performance (milligrams oxygen per hour per grams of plant wet wt) of *Egeria densa* plants immediately after four weeks in water (with chlorate and control) and after 6 h desiccation under different temperature regimens (see Table 2 for statistical details). Notice the two different *y*-axis scales.

DISCUSSION

The conditions considered in the design of these experiments were all parameterized using the evidence of environmental conditions, both climatic and of the amount of chlorate in the wetland that occurred during April and May 2004. Ever since the massive disappearance of *E. densa* from the wetland system during 2004, the suspicion that effluents from the Valdivia Paper Mill Plant were somehow responsible has been present [11], although this has never been proved, nor have the specific compounds within the effluents that could be toxic to *E. densa* been identified. In a previous study, Palma et al. [14] exhibited experimental evidence, also using a mesocosm approach, that the treated effluents do not have a negative impact on *E. densa*, although no specific compounds were tested in their experiments. The suspicion that chlorate could be the specific agent capable of this massive negative impact is not new and probably has to do with the fact that chlorate is employed in agriculture as an herbicide and defoliating agent [3]. However, the toxicity of chlorate to aquatic organisms has recently been reviewed, and based on the data available, chlorate was confirmed to be toxic to only a limited number of macro brown algae species and to some microorganisms [4]. In the case of *F. vesiculosus*, Rosemarin et al. [22] and Lehtinen et al. [23] found that the cellulose plant discharged effluents with an average concentration of 53 mg ClO_3^-/L to the Baltic Sea, whereas its average concentration in the receiving environment was approximately 0.5 mg/L during the discharge period. The toxic effect on the algae was reverted after further treatment of the effluent was implemented, reducing chlorate concentrations to less than 2 mg/L. Furthermore, these toxic effects have been suggested to be linked to the reduction of chlorate into the more toxic chlorite by enzyme systems that normally reduce nitrate [24]. Thus, its toxicity should decrease with increasing availability of nitrogen, nitrate being the most effective nutrient for protecting

Table 2. Two-factor analysis of variance repeated–measures mixed model for photosynthetic rates in plants before desiccation (exposed and not exposed [controls] to chlorate) and in the same plants after desiccation

Source of variation	SS	MS	*df*	*f*	*p*
Desiccation	148.0	74.0	2	20.32	<0.0001
Chlorate	1.811	1.811	2	0.5771	0.4693
Interaction	2.155	1.078	2	0.2959	0.7478
Subjects (matching)	25.10	3.138	8	0.8618	0.5662
Error	58.26	3.641	16		

SS = sum of squares; MS = mean square.

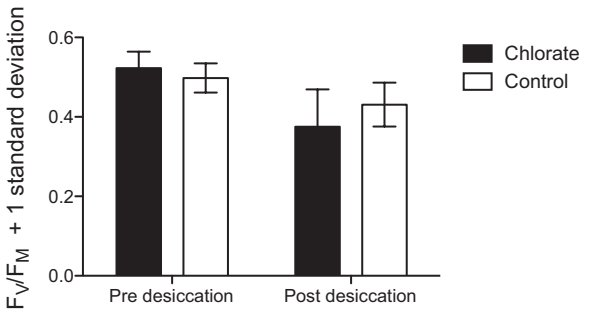

Fig. 4. Chlorophyll fluorescence of *Egeria densa* plants immediately after four weeks in water (with chlorate and control) and after 6 h desiccation at 60% humidity/20°C (see Table 3 for statistical details).

plants from chlorate toxicity [25]. However, in our experiment, nitrate concentrations were comparatively high, averaging 0.3 mg/L, and not significantly different from the values measured in 2004. Hence, nitrate protection would not explain the absence of chlorate toxicity in our experiment. None of the endpoints considered (morphological as well as physiologically related) in *E. densa* plants exhibited negative effects when compared with control plants. Therefore, we rule out that exposing *E. densa* plants to such concentrations of chlorate is detrimental. Similar conclusions were reached in a recent study in which plants were exposed to several different doses of chlorate for different periods inside a large mesocosm array, and several morphometric as well as physiologically related endpoints were quantified. The evidence suggests that *E. densa* is tolerant to fairly high levels of chlorate (i.e., median effective concentration in the order of 1,000 mg/L), at least three orders of magnitude larger than the highest concentration recorded in the wetland [15].

We found that desiccation is a major negative condition affecting *E. densa*, as have several authors before for this and other species [12,13,26]. Our results, however, reject the hypothesis stating that plants that had been exposed to the assumed chlorate levels present in 2004 are more susceptible to being negatively affected by desiccation than control plants under the assumed meteorological conditions in that area at that time. The temperature used during the desiccation phase of the experiments (5 or 20°C) was not a relevant factor in our results, which was also concluded by Bini and Thomaz [13] in a similar experimental approach. In addition to light and nutrients, desiccation is also an important determinant of plant growth and success of fragment development of aquatic plants in freshwater ecosystems [26]. This type of stress is important in reservoirs, where drawdown caused by dam operation is usual, and also in certain types of wetlands, where natural flood and drought pulses exist [26].

We conclude that under experimental conditions in a mesocosm system, *E. densa* is fairly resistant to exposures to relatively high levels of chlorate, whereas it is severely affected by desiccation conditions that lasted 6 h, corresponding to typical semi-diurnal tidal frequencies such as those existing in the wetland system, regardless of having been exposed to chlorate or not.

Table 3. Two-factor analysis of variance for F_v/F_m values in plants before desiccation (exposed and not exposed [controls] to chlorate) and in plants after desiccation for 6 h at 60% humidity/20°C

Source of variation	SS	MS	*df*	*f*	*p*
Desiccation	0.057	0.057	1	15.32	0.0012
Chlorate	0.001	0.001	1	0.3262	0.5758
Interaction	0.008	0.008	1	2.158	0.1612
Error	0.060	0.004	16		

F_v = variable fluorescence; F_m = maximum fluorescence; SS = Sum of squares; MS = mean square.

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REFERENCES

1. U. S. Environmental Protection Agency. 1999. *Alternative Disinfectants and Oxidants Guidance Manual*. EPA 815/R-99/014. Washington, DC.
2. MWH. 2005. *Water Treatment: Principles and Design*, 2nd ed. John Wiley & Sons, Hoboken, NJ, USA.
3. Logan B. 1998. A review of chlorate- and perchlorate-respiring microorganisms. *Bioremediation J* 2:69–79.
4. van Wijk DL, Hutchinson TH. 1995. The ecotoxicity of chlorate to aquatic organisms: A critical review. *Ecotoxicol Environ Saf* 32:244–253.
5. Rosemarin A, Lehtinen KJ, Notini M, Mattsson J. 1994. Effects of pulp mill effluent on Baltic Sea algae. *Environ Pollut* 85:3–13.
6. Coates JD, Michaelidou U, Bruce RA, O'Connor SM, Crespi JN, Achenbach LA. 1999. Ubiquity and diversity of dissimilatory (per) chlorate-reducing bacteria. *Appl Environ Microbiol* 65:5234–5241.
7. Schwarz AO, Urrutia H, Vidal JM, Perez N. 2012. Chlorate reduction capacity and characterisation of chlorate reducing bacteria communities in sediments of the Río Cruces Wetland in Southern Chile. *Water Res* 46:3283–3292.
8. Darling S, Côté M. 2008. Quantifying the evidence of ecological synergies. *Ecol Lett* 11:1278–1286.
9. Breitburg DL, Baxter JW, Hatfield CA, Howarth RW, Jones CG, Lovett GM, Wigand C. 1998. Understanding effects of multiple stressors: Ideas and challenges. In Pace ML, Groffman PM, eds, *Success, Limitations and Frontiers in Ecosystem Science*. Springer Press, New York, NY, USA, pp 416–431.
10. Yarrow M, Marín VH, Finlayson M, Tironi A, Delgado LE, Fischer F. 2009. The ecology of *Egeria densa* Planchon (Liliopsida: Alismatales): A wetland ecosystem engineer? *Rev Chil Hist Nat* 82:299–313.
11. Lagos NA, Paolini P, Jaramillo E, Lovengreen C, Duarte C, Contreras H. 2008. Environmental processes, water quality degradation, and decline of waterbird populations in the Rio Cruces wetland, Chile. *Wetlands* 28:938–950.
12. Marín VH, Tironi A, Delgado LE, Contreras M, Novoa F, Torres-Gómez M, Garreaud R, Vila I, Serey I. 2009. On the sudden disappearance of *Egeria densa* from a Ramsar wetland site of Southern Chile: A climatic event trigger model. *Ecol Model* 220:1752–1763.
13. Bini L, Thomaz S. 2005. Prediction of *Egeria najas* and *Egeria densa* occurrence in a large subtropical reservoir (Itaipu Reservoir, Brazil-Paraguay). *Aquat Bot* 83:227–238.
14. Palma AT, Silva M, Muñoz C, Cartes C, Jaksic F. 2008. Effect of prolonged exposition to pulp mill effluents on the invasive aquatic plant *Egeria densa* and other primary producers: A mesocosm approach. *Environ Toxicol Chem* 27:387–396.
15. Palma AT, Schwarz AO, Fariña JM. 2012. Experimental evidence of the tolerance to chlorate of the aquatic macrophyte egeria densa in a ramsar wetland in southern Chile. *Wetlands* DOI: 10.1007/s13157-012-0358-9.
16. Dere S, Günes T, Sivaci R. 1998. Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Turk J Bot* 22:13–17.
17. Lichtenthaler HK, Wellburn AR. 1985. Determination of total carotenoids and chlorophylls A and B of leaf in different solvents. *Biol Soc Trans* 11:591–592.
18. Demmig-Adams B, Adams WW III. 1992. Photoprotection and other responses of plants to high light stress. *Ann Rev Plant Physiology Plant Mol Biol* 43:599–626.
19. Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence: A practical guide. *J Exp Bot* 51:659–668.
20. Jimenez MS, Gonzalez-Rodriguez AM, Morales D, Cid MC, Socorro AR, Caballero M. 1997. Evaluation of chlorophyll fluorescence as a tool for salt stressed detection in roses. *Photosynthetica* 33:291–301.
21. Nairn RB, Lu Q, Fournier CP, Pantoja C. 2009. Three dimensional hydrodynamic numerical modelling of the Cruces River and the Carlos Anwandter Sanctuary, Valdivia, Chile. *Proceedings*, 8th International

Conference on Hidroinformatics, Concepción, Chile January 12–16, 2009, pp 1048–1056.
22. Rosemarin A, Mattsson J, Lehtinen KJ, Notini M, Nylén E. 1986. Effects of pulp mill chlorate (ClO_3^-) on *Fucus vesiculosus*: A summary of projects. *Ophelia* 4:219–224.
23. Lehtinen JJ, Motini M, Mattsson J, Landner L. 1988. Disappearance of bladder-wrack (*Fucus vesiculosus*) in the Baltic Sea: Relation to pulp-mill chlorate. *Ambio* 17:387–393.
24. van Wijk DJ, Kroon SGM, Garttener-Arends ICM. 1998. Toxicity of chlorate and chlorite to selected species of algae, bacteria and fungi. *Ecotoxicol Environ Saf* 40:206–211.
25. Guy M, Zabala G, Filner P. 1988. The kinetics of chlorate uptake by XD tobacco cells. *Plant Physiol* 86:817–821.
26. Silveira MJ, Thomaz SM, Mormul RP, Pereira-Camacho F. 2009. Effects of desiccation and sediment type on early regeneration of plant fragments of tree species of aquatic macrophytes. *Int Rev Hydrobiol* 94:169–178.