RESEARCH PAPER

Chemical repellency and adverse effects of free-floating macrophytes on the cladoceran *Ceriodaphnia dubia* under two temperature levels

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Abstract Macrophytes can structure biological assemblages in subtropical lakes. Zooplankton often uses them as a refuge, but in the absence of predators many species frequently avoid them. It is hypothesized that plant exudates can act as "repellent factors" because they can be detrimental to the animals' life history. This work aimed to test both assumptions: whether exudates of two free-floating species, *Eichhornia crassipes* and *Salvinia biloba*, repel the cladoceran *Ceriodaphnia dubia* and whether they affect its life history. Furthermore, given the possibility that increasing temperatures can modify the observed responses, the experimental research was carried out at two temperature levels: 21 and 25 (±1) °C. First, an avoidance experiment was performed inside narrow aquaria containing the plants, so that repellence was analyzed according to the location of animals after 30, 60 and 90 min. Second, for the life cycle experiments, cladocerans were raised in macrophyte-conditioned water and their lifespan, growth and reproduction were daily monitored. Macrophytes' exudates effectively repelled *C. dubia* and affected its life history by decreasing its life expectancy, increasing the age at first reproduction and lowering the number of eggs per female. The magnitude of such effects depended on the treatment, i.e. both plants together caused more negative effects than *E. crassipes* alone, and this latter was more negative than *S. biloba*. At higher temperature, the negative effects were intensified but *C. dubia* maintained the same order of sensitivity to each treatment. This suggests that temperature variations, within a tolerance range, do not alter the allelopathic interactions between the studied species.

Introduction

In subtropical shallow lakes, it is likely that vegetated littoral zones support the highest heterogeneity of ecological niches, leading to the development of complex biological structures (Jeppesen et al. 1998). In those littoral environments, submersed and free-floating macrophytes are of particular interest for limnologists, due to both their abundance and their role in structuring different inhabiting communities such as zooplankton (Moss et al. 1997; Meerhoff et al. 2007).

Field and laboratory studies have suggested that macrophytes provide a spatial refuge against predators (Timms and Moss 1984; Moss et al. 1994; Harrison et al. 2005; Cazzanelli et al. 2008; González Sagrario and Balseiro 2010; Van Onsem et al. 2010) which, in mesotrophic and eutrophic systems may contribute to the stabilization of an 'alternative' clear water state (Scheffer et al. 1993). Conversely, it has also been demonstrated that macrophytes create 'dangerous environments' because they shelter voracious predators like macroinvertebrates and fish larvae (Lauridsen and Lodge 1996; González Sagrario and Balseiro 2010). Furthermore, the repellent factor has also been associated with structural interference since submersed stems and roots can inhibit swimming or feeding activities of some large cladocerans, leading to a reduction

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of their growth or reproduction (Burks et al. 2000; Cerbin et al. 2007; Higuti et al. 2010; González Sagrario and Balseiro 2010).

Even though early publications have documented the repellency of macrophytes to some planktonic organisms through chemical exudates (Pennak 1973, Dorgelo and Heykoop 1985), little attention has been given to this phenomenon as an important mechanism for the structuring of such biological assemblages (Lampert and Sommer 1997; van Donk and van de Bund 2002). In this regard, it has been demonstrated that allelopathy is a powerful strategy for macrophytes to compete with nearby autotrophs or to face herbivory (Mulderij et al. 2005; Gross et al. 2007; Macías et al. 2008), but the released compounds can be detrimental for the life history of non-target organisms such as fishes (Applebaum and Birk 1979; Temmink et al. 1989; Agostinho et al. 2007), tadpoles (Maerz et al. 2005), rotifers (Hasler and Jones 1949), and mysids (Lindén and Lehtiniemi 2005), among other animals (Dhillon et al. 1982; Johnson and Mulla 1983).

However, the relative importance of such cues to planktonic animals is still not understood and most studies are mainly concerned with submersed macrophytes, especially those belonging to the genus *Miriophylum* and *Elodea*, whose allelopathic properties are well-known (Lauridsen and Lodge 1996; Burks et al. 2000). With some exceptions, little attention has been paid to the repulsive effects of the free-floating plants, although they are highly representative in temperate shallow lakes and play key roles in ecological interactions (Agostinho et al. 2007). A contribution of Meerhoff et al. (2003) reports strong evidence that chemical cues exuded by free-floating plants of the genera *Eichhornia*, *Salvinia* and *Pistia* are capable of repelling the *Daphnia obtusa* water flea. However, it is likely that this phenomenon is species-specific and varies with environmental conditions (Moss et al. 1997; Scheffer et al. 1993). Many stress situations like low light intensity, dissolved oxygen, herbivory and high temperatures could be determining factors in the release of chemical cues (Sharkey and Singaas 1995; Gross 1999; Ervin and Wetzel 2003). In this context, the effects of macrophyte exudates under different climate conditions is an issue of increasing importance, particularly when considering the potential impact of global warming on aquatic systems (Burks et al. 2006).

Taking into account the above mentioned considerations, the aim of this work was two-fold: to analyze whether exudates of two (isolated or jointed) free-floating species, *Eichhornia crassipes* (water hyacinth) and *Salvinia biloba* (water fern), repel the cladoceran *Ceriodaphnia dubia* and, considering that the repellency may suggest the existence of a negative factor in the surrounding environment, to test whether such exudates actually affect its life history. Furthermore, since it has been suggested that

environmental variables such as increasing temperatures can modify the observed responses, the experiments were carried out at two temperature levels for comparison. We hypothesized that chemical exudates released by plants repel the cladocerans, cause important biological costs by affecting their life history and increasing temperature, 'speed up' the avoidance response, and intensify its negative effects on the life history of cladocerans.

Methods

Macrophyte culture

The free-floating species *Eichhornia crassipes* and *Salvinia biloba* were selected since previous field studies documented their notorious high abundance in many shallow neotropical freshwater environments, eventually forming large floating sheets (Agostinho et al. 2007). Furthermore, it has been observed that lower densities of planktonic crustaceans occur under free-floating plants, particularly under *Eichhornia* sheets (Brendonk et al. 2003; Meerhoff et al. 2007), which suggests their negative effect on the organisms.

One week before the start of the experiment, several plants of both species (*E. crassipes* and *S. biloba*) were collected from different shallow lakes of the alluvial plain of the Paraná river and transferred to the laboratory for their acclimation to a constant photoperiod (16:8 h light: dark cycle) and temperature conditions (21 ± 1 or 25 ± 1 °C, depending on the experiment). The two selected temperature levels were in the range of the temperatures registered in winter and summer in natural aquatic systems (Devercelli 2009; José de Paggi et al. 2012).

In the laboratory, the roots were carefully washed with aged tap water to remove epiphyton. For maintenance, each species was transferred separately to different plastic aquaria (length: 30 cm; height: 25 cm; width: 40 cm) filled with filtered (50 μm) and autoclaved pond water: nitrates: <0.1 mg l^{-1}; nitrites: 0.01 mg l^{-1}; ammonium: 0.29 mg NH_3 l^{-1}; chlorides: 3.5 mg l^{-1}; sulphates: 8.3 mg l^{-1}; total alkalinity: 77 mg $CaCO_3$ l^{-1}; bicarbonates: 94 mg l^{-1}; sodium: 7.7 mg l^{-1}; magnesium: 6.8 mg l^{-1}; calcium: 12.9 mg l^{-1}; potassium: 1.8 mg l^{-1}. Since light intensity was a limiting factor for plant maintenance, an additional fluorescent lamp of white cold light (3593.3 ± 77 Lux) was located above the area occupied by the aquaria, imitating the above light incidence of a natural aquatic environment. This procedure allowed us to maintain the plants in good conditions for several weeks.

During maintenance, the culture medium was partially renewed (50 %) twice a week and the decomposed material was extracted daily. A permanent aereation of each

aquarium was provided by electric aerators. pH and conductivity were controlled weekly using a calibrated HANNA water analyzer.

Macrophyte-conditioned water

For the behavioral and life-cycle experiments with *C. dubia*, the used control medium was aged (24 h) tap water (hereafter, Control treatment) since this was the same in which cladocerans were reared and maintained for several generations in the laboratory (pH: 8.39 ($\pm$0.24); conductivity: 245.33 ($\pm$28.18) μS/cm.).

To obtain plant exudates, the tap water (Control) was conditioned in the presence of *Eichhornia* alone (*Eichhornia* treatment), *Salvinia* alone (*Salvinia* treatment) or *Eichhornia* and *Salvinia* together (E+S treatment). For the *Eichhornia* treatment, one acclimated plant of *E. crassipes* (11.13 $\pm$ 1 SD g dry weight), from the stock culture, was transferred to a 6 l tank with control water. For the *Salvinia* treatment, acclimated plants (2.6 $\pm$ 0.4 SD g dry weight) from the stock culture were transferred to a 2 l tank with control water. For the E+S treatment, acclimated plants of each species (14.43 $\pm$ 1.9 SD g total dry weight) were transferred to a 6 l tank with control water. As a result of such procedure, the total biomass of each treatment was 1.86 ($\pm$0.17 SD) g l^{-1} for the *Eichhornia* treatment; 1.3 ($\pm$0.21 SD) g l^{-1} for *Salvinia* treatment; 2.41 ($\pm$0.33 SD) g l^{-1} for E+S treatment. The statistical analysis showed that there were no significant differences between *Eichornia* and *Salvinia*, nor between *Eichornia* and E+S biomasses (Dunn's Multiple Comparisons Test $U = 3.33$ and $U = -2.33$, respectively; $p > 0.05$ in both cases). However, there were statistical differences between *Salvinia* and E+S biomasses (Dunn's Multiple Comparisons Test $U = -5.67$, $p < 0.05$). Immediately before use, the conditioned water was filtered using Whattman GF/F filter papers.

During the experiments, the water and all plants were renewed every 4–5 days to avoid plant degradation or alterations of the water chemical quality. In all cases, new shoots were used when renewing the plants and three replicates were carried out for each experiment. Each aquarium was maintained under the same light intensity and photoperiod as mentioned for the macrophyte stock cultures. The same procedure was replicated at 21 $\pm$ 1 and at 25 $\pm$ 1 °C, for each experiment.

Ceriodaphnia dubia stock culture

Cladocerans were collected with a plankton net (100 μm) from wetlands belonging to the Paraná alluvial plain and cultured in the laboratory during several generations under constant photoperiod (16:8 h light: dark) and temperature

(21 $\pm$ 2 °C) conditions in glass containers, in a density of 50 ind.l^{-1}. For culture medium, dechlorinated and well-aerated (for 48 h) tap water was used (Control medium).

The organisms were fed daily with *Chlorella vulgaris* (algal density: 3.7 $\times$ 10^5 cel ml^{-1}). The algae were cultured using Bold Basal Medium (BBM) (Sager and Granik 1953) under sterile conditions. Then, they were concentrated by centrifugation at 500 rpm for 7 min and resuspended in the same medium used for the cladocerans, with gentle shaking. The algal quantification (ind. ml^{-1}) was determined using an inverted Wild microscope at 400X following the Utermöhl (1958) method.

Avoidance experiments

Avoidance experiments were performed inside narrow glass aquaria (length: 40 cm; high: 20 cm; wide: 12 cm) filled with 7 l of control water. Each aquarium was externally divided into four imaginary compartments of 10 cm (length) each (C1, C2, C3 and C4).

For macrophyte treatments, one acclimated plant of *E. crassipes* 1.8 ($\pm$0.3 SD) g l^{-1}; some acclimated plants of *Salvinia* 1.17 ($\pm$0.2 SD) g l^{-1} or both acclimated species together 2.67 ($\pm$0.22 SD) g l^{-1} were located in the first compartment of the aquarium. The compartment occupied by the plants was separated from the rest of the compartments by means of an opaque mesh, so that the cladocerans could only detect the presence of the plants through their 'odor' (but not by visual or mechanical signals) (Fig. 1). The purpose of this separation was to avoid the structural effects of roots.

For the controls, the first compartment (C1) remained without plants but it was also separated by the opaque mesh
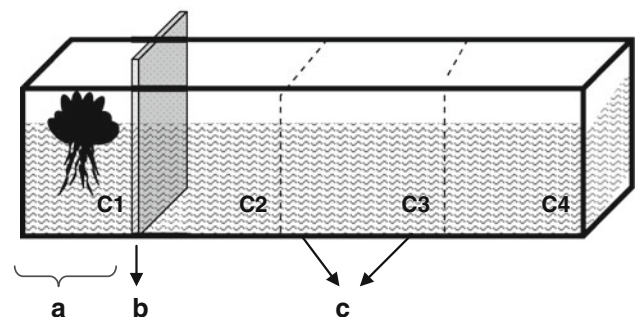

Fig. 1 Diagram of the experimental design for the avoidance experiments with *Ceriodaphnia dubia* and free-floating macrophytes. The figure represent the aquarium (length: 40 cm; high: 20 cm; wide: 12 cm) filled with 7 l of control water. It was separated into four compartments of 10 cm with external pointed lines. The macrophytes were located in the first compartment, *C1* (*a*) which was separated from the other compartments (*C2*, *C3*, *C4*), where cladoceran were located, with an opaque mesh (*b*). *Pointed lines* (*c*) represent the dividing lines that separate each aquarium's compartment

from the other compartments to maintain similar conditions as for the treatments with plants.

At the start of the experiments, twenty cladocerans (adults) were introduced into each aquarium. Control and treatments with *Eichhornia*, *Salvinia*, or E+S were triplicated consecutively and carried out in two different acclimated rooms: at 21 (±1) and 25 (±1) °C. Light intensity was maintained constant at 3593.3 ± 77 Lux.

In order to estimate the avoidance of organisms from plants, the number of animals present in each subsequent compartment of the aquaria was quantified three times: at 30, 60 and 90 min, following Burks et al. (2001). The relative proportion of individuals in each aquarium compartment was calculated in relation to the total of individuals observed. The Chi square goodness of fit test for more than two categories was used to compare their location between control and treatments, in each observation (30; 60; 90 min), and between temperatures (21 and 25 °C).

The two-way repeated measure ANOVA (ANOVA RM) was employed to evaluate the significance of treatments (Control, *Eichhornia*, *Salvinia* and E+S) on the location of *C. dubia* with time. Tukey's post hoc test was used for comparisons among treatments. The normal distribution of data, homoscedasticity and sphericity were verified using the Kolmogorov–Smirnov, Levene and Mauchly test, respectively. In all cases, differences were considered significant at values of $p < 0.05$.

Life cycle experiments

Ovigerous females of *C. dubia* were isolated from the stock culture to obtain newborn (neonates, <24 h). The neonates were placed individually into glass containers with 60 ml of filtered control or macrophyte-conditioned water (*Eichhornia*, *Salvinia*, or E+S). Twelve replicates per control and each treatment were carried out using the same generation of neonates. During the experiments, culture media were renewed daily and the organisms were fed daily with *Chlorella* sp. in a concentration of 3.7×10^5 cells ml^{-1}, as described for the stock culture.

The effects of the macrophyte exudates (*Eichhornia*, *Salvinia*, or E+S) on lifespan (longevity), growth and reproduction of *C. dubia* were daily monitored throughout its life cycle. As indicator of growth, the total length (from the top of the head to the tip of the spine at the posteriordorsal angle of the carapace) of each individual was measured three times: as a newborn, at the age of first reproduction and when the animal died, using an ocular micrometer Nikon 41602 under a compound binocular microscope. The reproductive traits analyzed were: number of eggs per female (eggs/female), age of first reproduction (AFR) and number of clutches per female (Clutches). This

experiment was carried out simultaneously at 21 ± 1 and at 25 ± 1 °C inside two incubator chambers Semedic I-290P for comparisons.

To test the significance of the temperature conditions (21 and 25 °C) and each water treatment (Control, *Eichhornia*, *Salvinia*, or E+S) on the lifespan and reproductive traits (eggs/female, AFR and RE) of *C. dubia*, a two-way ANOVA was used, followed by a Tukey's post hoc test for comparisons among treatments. Differences were considered significant at $p < 0.05$. Prior to ANOVA, data were tested for normality (Kolmogorov–Smirnov's test) and homogeneity of variances (Levene's test).

The two-way repeated measure ANOVA (ANOVA RM) was employed to analyze the significance of temperature or water treatment on the growth of *C. dubia*. Prior to this test, the normal distribution of data (Kolmogorov–Smirnov test), homoscedasticity (Levene's test) and sphericity (Mauchly's test) was verified. The within-subject factors of the ANOVA RM were time (3 levels) and between-subjects factors were water treatment (Control, *Eichhornia*, *Salvinia*, or E+S) or temperature conditions (21 and 25 °C). Differences were considered significant at $p < 0.05$.

Results

Avoidance experiments

Overall, the location of organisms did not vary with time at 21 °C (ANOVA RM: $n = 12$; $df = 3$; C2: $F = 1.29$ $p = 0.302$; C3: $F = 2.31$ $p = 0.131$; C4: $F = 3.11$ $p = 0.072$). However, at 25 °C cladocerans were more active, showing high variations throughout all periods of observation (ANOVA RM: $n = 12$; $df = 3$; C2: $F = 22.3$; C3: $F = 46.002$; C4: $F = 29.7$, $p < 0.001$ in all cases).

Under both temperature conditions (21 and 25 °C), in the control aquaria (without macrophytes), the percentage of cladocerans in the second compartment (C2, beside the isolated compartment) was always higher than in the aquaria with macrophytes (Fig. 2). In several occasions, it was observed that the organisms from the control aquaria were notoriously swimming close to the spacer mesh but, despite its not being quantified, this behavior was not observed in the other treatments.

At 21 °C, cladocerans were clearly repelled by *E. crassipes* and the macrophytes together (S+E) as, in the proximal compartment (C2), the percentage of organisms from the control aquaria significantly overcame that of the mentioned treatments (ANOVA RM, $df = 3$; $F = 13.006$, $p < 0.01$; Tukey, $p = 0.002$ and $p = 0.015$, respectively). Furthermore, in compartment 4 (the farthest one) of the *Eichhornia* treatment, the percentage of organisms was
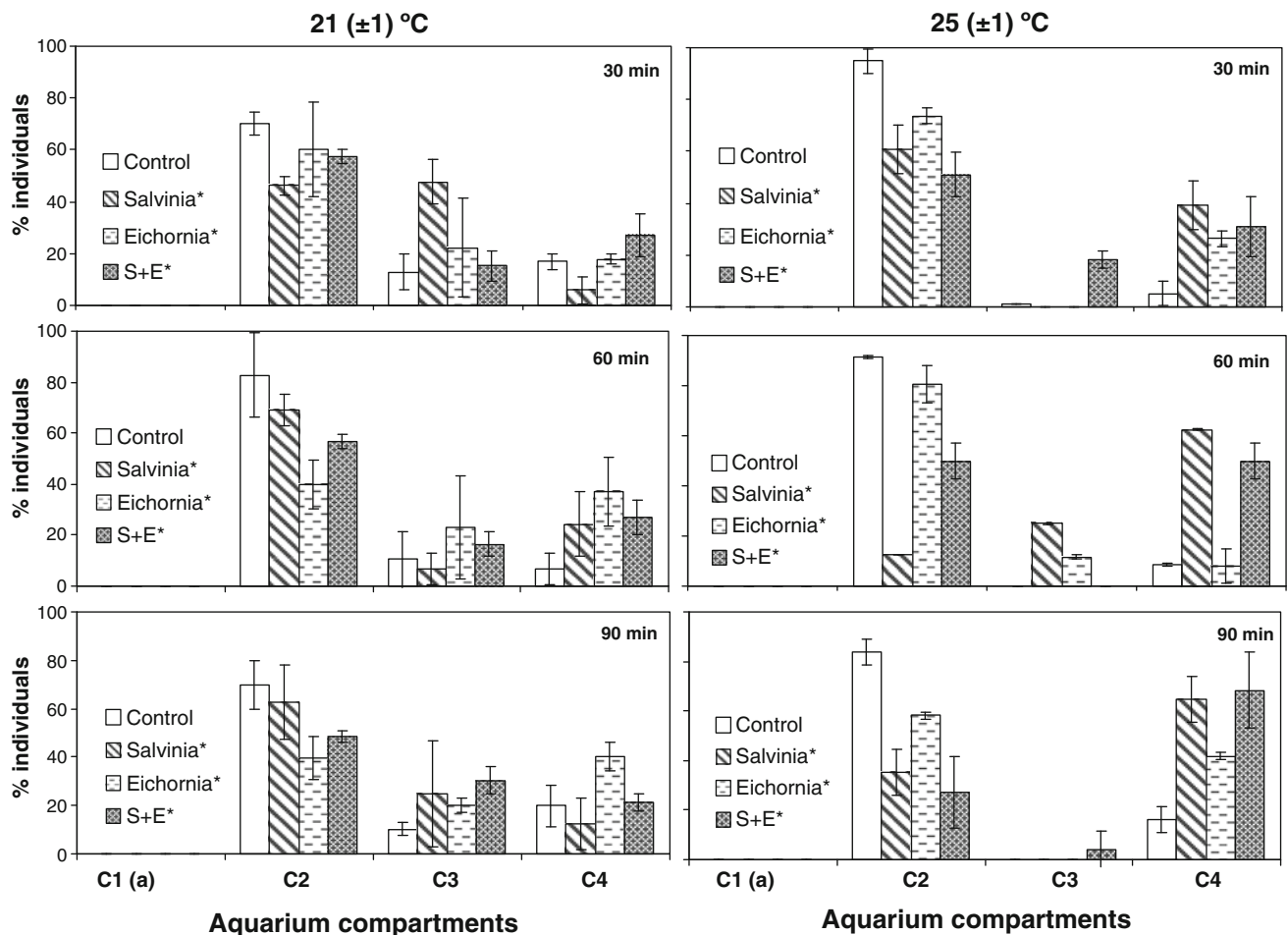
Fig. 2 Location of *Ceriodaphnia dubia* in the aquaria, in relation to the first compartment (*C1a*) without macrophytes (Control), with *Salvinia biloba* (Salvinia), with *Eichorina crassipes* (Eichornia) or with *S. biloba* and *E. crassipes* together (S+E). In each aquarium, *C1* was separated from the following aquarium compartments (*C2*, *C3*, *C4*) with an opaque mesh so the animals could perceive the plants only through chemical signals, but not visually. The figure shows the percentage of cladocerans in *C2*, *C3* and *C4* (Mean and $\pm$ SD, $n = 3$) at two temperature conditions: 21 ± 1 °C (*left panels*) and 25 ± 1 °C (*right panels*). (*) Significant differences between each treatment (Salvinia, Eichornia or S+E) and Control, based on Chi square goodness of fit test ($n = 6$). **a** Compartment isolated, with or without plants, depending on the treatment

higher than in those of the control aquaria after 90 min of exposition (Tukey, $df = 3$; $p = 0.021$).

Even though at 25 °C the animals location was more polarized than at 21 °C (i.e. cladocerans were concentrated in both extremes), important differences were registered between treatments in all compartments (ANOVA RM, $df = 3$; C2: $F = 98.44$; C3: $F = 17.8$; C4: 60.98, $p < 0.01$ in all cases). Such differences show that *C. dubia* was repelled by the three treatments with macrophytes, as in C2 the percentage of cladocerans was higher in the control treatment than in *Eichhornia*, *Salvinia* or S+E treatments (Tukey, $df = 3$; $p < 0.01$ in all cases). Moreover, in C3 and C4, significant differences between Control and *Eichhornia*, *Salvinia* or S+E treatments were also found (Tukey, $df = 3$; $p < 0.01$) (Fig. 2).

Life-cycle experiments

Longevity of *C. dubia* from the control water was higher at 21 °C (40 ± 10 days) than at 25 °C (23 ± 4 days), this difference being highly significant (ANOVA, $n = 96$, $df = 1$; $F = 168.8$, $p < 0.01$) between the two temperature conditions. Cladoceran longevity decreased progressively when they were exposed to exudates of *Salvinia*, *Eichhornia* and both macrophytes together (S+E) respectively, and this pattern was similar at 21 and 25 °C (Fig. 3). The interaction (temperature × treatment) was also very significant (ANOVA, $n = 96$, $df = 3$; $F = 6.72$, $p < 0.01$) and, within each temperature level, all treatments with the macrophyte exudates were significantly different from the respective control (Fig. 3).

The age at first reproduction (AFR) was the least variable reproductive attribute of *C. dubia* since it only showed significant differences at 25 °C between cladocerans from the control group (7.92 ± 1.5 days) and those exposed to *Eichhornia* exudates (10.3 ± 2.4 days). These latter organisms clearly delayed their sexual maturity by approximately 2.5 days (ANOVA: $n = 96$, $df = 3$; $F = 4.88$, $p = 0.007$) (Table 1).

The number of eggs per female (Eggs/female) was higher at 21 °C than at 25 °C in all treatments (ANOVA: $n = 96$, $df = 3$; $F = 368$, $p < 0.01$) (Table 1). At 21 °C, Eggs/female were significantly lowered only with the exudates of both macrophytes, S+E (Tukey, $F = 52.68$; $p < 0.01$). However, at 25 °C, all treatments (*Salvinia*, *Eichhornia* and S+E) reduced the number of offspring, in comparison with the control ($F = 31.08$, $p < 0.01$).

The total number of clutches (Clutches) of cladocerans in all treatments with exudates was very low, both at 21 and 25 °C (Table 1), when compared with the controls. In this case, significant differences were registered among temperatures ($F = 161.58$, $p < 0.01$), treatments

Fig. 3 Mean (±SD) longevity (in days) of *Ceriodaphnia dubia* raised in control water (Control) and treatments with *Salvinia* (Sal), *Eichornia* (Eich) or *Salvinia* and *Eichornia* (S+E) exudates at two temperature levels: 21 °C (*dark bars*) and 25 °C (*clear bars*). *Different letters*, within each temperature condition, indicate significant differences between treatments based on Tukey's multiple comparison tests

($F = 54.7$, $p < 0.01$) and the interaction between these two factors ($F = 9.19$, $p < 0.001$).

At 21 °C, *C. dubia* exposed to S+E treatment was significantly smaller than the cladocerans from the control ($F = 4.2$, $p < 0.01$). However, no significant differences in their length were observed among control and the other treatments (*Salvinia* and *Eichhornia*) at the same temperature conditions (Fig. 4). Similarly, no significant differences in their body length were observed at 25 °C among treatments (Control, *Salvinia*, *Eichhornia* or S+E) ($F = 1.27$, $p = 0.27$).

Discussion

The present work showed that the chemical exudates released by the two studied free-floating species, *E. crassipes* and *S. biloba*—isolated and together—repelled *C. dubia* since cladocerans exposed to plants in the aquaria remained, on average, in the farthest compartment from the organisms from the control aquaria. The negative effects of such exudates (and the possible cause of the observed evasion) also imply biological costs for cladocerans by altering several life cycle parameters such as lifespan, age at first reproduction, eggs per female and number of clutches.

The observed avoidance behavior of *C. dubia* from *Eichhornia* and *Salvinia* is in agreement with previous experimental investigations which reported that the cladoceran *Daphnia magna* actively avoids the submersed macrophytes *Myriophyllum* and *Elodea,* in the absence of fishes (Pennak 1973; Lauridsen and Lodge 1996; Burks et al. 2001). Likewise, this evasion phenomenon is also consistent with field recordings in subtropical lakes which demonstrate that most planktonic organisms avoid the shore, remaining in open waters, despite the presence of possible predators (González Sagrario and Balseiro 2010).
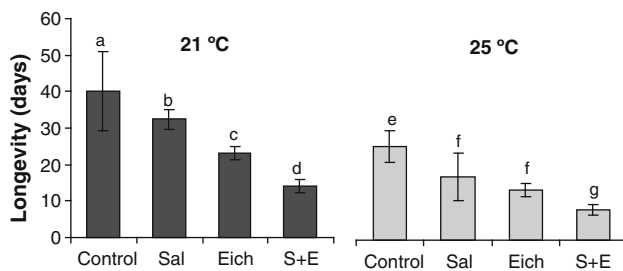
Table 1 Mean (±SD) reproductive parameters of *Ceriodaphnia dubia* raised in control water (Control) and treatments with *Salvinia* (Salvinia), *Eichorina* (Eichornia) or *Salvinia* and *Eichornia* (S+E) exudates at two temperature levels: 21 and 25 °C

	Control	Salvinia	Eichornia	S+E
21 °C				
AFR	7.25 (±2.4)	7 (±0.8)	8.67 (±0.9)	8.55 (±1.4)
Eggs/Female	32.33 (±11.4)	26 (±5.6)	35.9 (±5.4)	4.08 (±2.2)**
Clutches	11.08 (±4.2)	8.33 (±1.7)**	6.83 (±1.2)**	1.92 (±0.9)**
25 °C				
AFR	7.92 (±1.5)	8.9 (±1.7)	10.3 (±2.4)**	6.67 (±0.8)
Eggs/female	9.83 (±3.1)	5.42 (±4.3)**	0.67 (±0.9)**	0.93 (±0.8)**
Clutches	5.08 (±1.6)	2.25 (±1.8)**	0.5 (±0.5)**	1.0 (±1.4)**

Double asterisks indicate significant differences with control at $p < 0.01$ (Tukey's multiple comparison test)

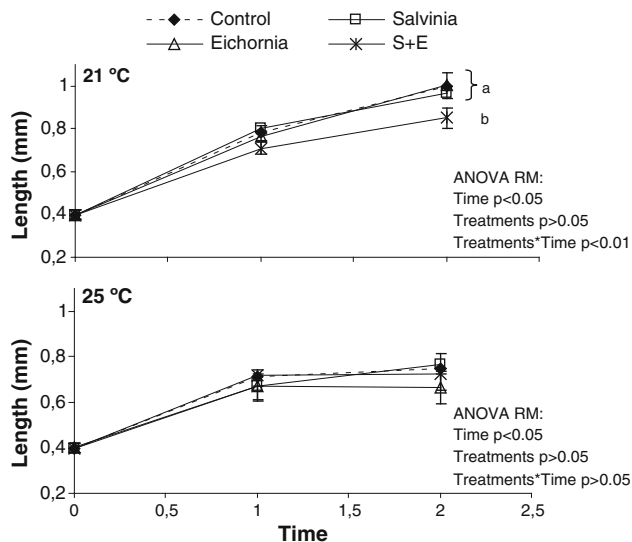*AFR* age at first reproduction, *Eggs/female* number of eggs per female, *Clutches* number of clutches per female

Fig. 4 Mean (±SD) length (in mm) of *Ceriodaphnia dubia* raised in control water (Control) and treatments with *Salvinia* (Salvinia), *Eichorina* (Eichornia) or *Salvinia* and *Eichornia* (S+E) exudates at two temperature levels: 21 and 25 °C. *p* values are from ANOVA RM and *different letters* indicate significant differences between treatments based on Tukey's multiple comparison test

In this line, other studies performed on wetlands mainly colonized by *Eichhornia*, also demonstrated that cladocerans were more abundant in central zones than in the littoral ones (where *Eichhornia* remained), suggesting that allelopathic substances exuded by this species could be potentially 'dangerous' for planktonic organisms (Meerhoff et al. 2003).

Although previous investigations showed that certain planktonic organisms avoid free-floating plants more than submersed macrophytes (Meerhoff et al. 2007), unfortunately there are still few experimental investigations that effectively demonstrate the importance of chemical communication promoting such behavior. Moreover, most studies emphasize the importance of the 'shading' effects, root architecture or the associated predators as the main factors of the repellency (Pennak 1973; Meerhoff et al. 2007; González Sagrario and Balseiro 2010). However, the results of this work corroborate that the released exudates also have high importance and effectiveness in such phenomena, thus contributing to the hypothesis that suggests that macrophytes are a dangerous place rather than a refuge for the organisms, especially in subtropical shallow lakes (Lauridsen and Lodge 1996; Meerhoff et al. 2007, González Sagrario and Balseiro 2010).

Furthermore, it can be suggested that exudates can act both directly, by affecting the organisms' fitness—as will be discussed later—or indirectly, as an 'alarm signal' by anticipating the reduced availability of algae and microorganisms (i.e. food) in the surrounding environments (Sinistro et al. 2006; Tezanos Pinto et al. 2007). Even though further research is needed, it is plausible to think that an early recognition (through chemical signals) of the availability of food in the surrounding environment might be the result of complex coevolutive processes among macrophytes and aquatic organisms, in which these latter ones are favored.

When cladocerans were raised with the different macrophyte exudates, their lifespan clearly decreased. The negative effects followed a similar pattern both at 21 and at 25 °C: the exudates of *E. crassipes* were more detrimental than those of *S. biloba*, but those of the macrophytes together produced the most negative effect. It is important to state here that the differences in the cladocerans responses among treatments should not be necessarily attributed to a difference between plant species (i.e. nature of allelochemicals) but also to a difference in biomasses (i.e. quantity of allelochemicals), since biomass of E+S treatment was higher than that of *Salvinia* and slightly higher than that of *Eichhornia* treatments. On the other hand, the enhancement of the negative effects of both plants together could be closely related to the competition between them in the aquarium, since it has been recognized that aquatic plants are able to release higher allelopathic chemicals when they are surrounded by other autotrophs (macrophytes and phytoplankton) (Gross 1999; van Donk and van de Bund 2002; Ervin and Wetzel 2003).

Despite the fact that age at first reproduction (AFR) of *C. dubia* was not modified, cladocerans exposed to the macrophyte exudates (together at both temperatures or isolated at 25 °C) reduced their ovipositions as well as the number of clutches. Such responses resemble those registered by *D. magna* exposed to exudates of *Elodea canadensis* (Burks et al. 2000) and *Myriophyllum verticillatum* (Cerbin et al. 2007) and might represent a negative effect more than an adaptative strategy to variable environmental conditions. Such effects might imply a minor participation of genes to future generations and, in the long term, it can cause serious damage to a population (Cerbin et al. 2007).

The absence of alteration in cladoceran growth, particularly at 25 °C, could be associated with the fact that high temperatures can increase metabolism, thus favoring higher food assimilation than at low temperatures. The advantages of such metabolic variations at different temperatures have also been recognized in the diel vertical migration strategy (DVM) of certain planktonic organisms (Lampert 2005). On the other hand, it has been suggested that alteration in growth could be associated with the plant architecture more than with their allelopathic substances (Cerbin et al. 2007). This is because complex architectures can involve a high energy cost (i.e. higher intensity of swimming maneuvers) and, on some occasions, low food availability for the inhabitant organisms.

The substances in the surrounding environment (termed sensu lato exudates) that induced the observed avoidance of *C. dubia* and also caused alterations in their life history were not detailed in this work. Nevertheless, it is known that such substances constitute complex mixtures of secondary metabolites that plants often use as a chemical defense against herbivory or as a competition means (Ervin and Wetzel 2003).

Among them, saponins, tannins, phenols, terpens and alkaloids are the main compounds that, once released, can seriously affect non-target organisms such as fish, by lysing blood cells, or other microorganisms, by altering their physiological activities (Applebaum and Birk 1979; Levin 1971; Macías et al. 2008). Although the alellopathic potentiality of the macrophytes used in this study requires much further investigation, the experimental analysis demonstrated that the richness of invertebrates associated with the decomposition of *Eichhornia azurea* increased as poliphenols decrease (Stripari and Henry 2002). In addition, it is well-known that *Eichhornia* is an invasive species worldwide; however, the local fauna (poorly adapted to its presence) able to use its root as an ecological niche is frequently scarce (Higuti et al. 2010). On the other hand, phytochemical studies with a species of *Salvinia* also demonstrate that it can produce flavonoids, tannins, saponins, esteroids and phenols, which inhibit the growth of bacteria, insects and fungi (Mithraja et al. 2011). All these studies suggest the high allelopathic ability of the macrophytes analyzed here and, at the same time, they reinforce the hypothesis about their negative effects to planktonic cladocerans such as *C. dubia*.

Finally, it was clearly recognized that the higher temperature (25 °C) intensifies the negative effects of plant exudates on *C. dubia* life cycle. This is in agreement with previous observations reported by Pennak (1973), who recognized that *Daphnia* was more sensitive to hydrophytes at the highest analyzed temperature. We can argue that such intensifications must be related to higher metabolic processes both in cladocerans ('speeding up' the response or favoring the intake of toxic chemicals) as well as in plants (favoring the release of exudates). However, the similarity in the patterns observed (avoidance and life-cycle) in both analyzed temperatures suggests the independence of this parameter in the interactions among the studied macrophytes and cladocerans, within a certain tolerance range. This is also in agreement with Burks et al. (2001), and González Sagrario and Balseiro (2010), who suggested that environmental variables such as temperature, DO, pH and light intensity do not influence the mechanisms of 'habitat choice'.

Although the obtained results can hardly be directly extrapolated to nature, they allow us to better understand the biological interaction among free-floating macrophytes and zooplankton, and contribute to the hypothesis about their importance as structuring factors of biological assemblages in nature (through chemical and structural mechanisms).

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