

An experimental study of habitat choice by *Daphnia*: plants signal danger more than refuge in subtropical lakes

MARIANA MEERHOFF,^{*,†,‡} CLAUDIA FOSALBA,^{*} CARLA BRUZZONE,^{*} NÉSTOR MAZZEO,^{*} WILLEMJN NOORDOVEN[§] AND ERIK JEPPESEN^{†,‡}

^{*}Sección Limnología, Facultad de Ciencias, Universidad de la República. Iguá, Montevideo, Uruguay

[†]National Environmental Research Institute. Vejlshøvej, Silkeborg, Denmark

[‡]Department of Plant Biology, Institute of Biological Sciences, Aarhus University, Ole Worms Allé, Aarhus C, Denmark

[§]Department of Environmental Sciences, Aquatic Ecology and Water Quality Management Group, Wageningen University, Wageningen, The Netherlands

SUMMARY

1. In shallow temperate lakes, submerged plants often provide refuge for pelagic zooplankton against fish predation, a mechanism with potential strong cascading effects on water transparency and on the entire ecosystem. In (sub)tropical lakes, however, the interaction between aquatic plants and predation may be more complex, particularly because fish density is high within the plant beds in such systems.
2. Using laboratory 'habitat choice' experiments, we determined the effects of three (sub)tropical free-floating plants, *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia auriculata* and the cosmopolitan submerged *Ceratophyllum demersum*, on horizontal movement by the water flea *Daphnia obtusa*. We tested for avoidance of plants in the absence and presence of alarm signals from crushed conspecifics and chemical cues from the fish *Cnesterodon decemmaculatus*, the fish have been subjected to different feeding regimes.
3. In the absence of other stimuli, *D. obtusa* strongly avoided the plants and the crushed conspecifics, as expected. However, the response to fish was insignificant regardless of their previous feeding regime. The avoidance of free-floating plants was more pronounced than that of the submerged plant. Contrary to predictions based on research in temperate lakes, *Daphnia* did not take refuge among the plants but rather swam away from them when exposed simultaneously to plants and alarm signals.
4. We hypothesise that the avoidance of plants by *D. obtusa* may ultimately be attributable to an expectedly higher predation risk within the plants than in the pelagic, because of a high density of associated zooplanktivorous fish in the former. In the (sub)tropics, therefore, aquatic plants and particularly the free-floating ones, may not promote cascading effects via *Daphnia* grazing on phytoplankton as seen in temperate eutrophic lakes.

Keywords: alarm signal, chemical cue, kairomone, predator-avoidance, refuge effect

Correspondence: Mariana Meerhoff, Sección Limnología, Facultad de Ciencias, Universidad de la República. Iguá 4225, CP 11400, Montevideo, Uruguay.
E-mail: merluz@fcien.edu.uy

Introduction

Predation by fish on pelagic zooplankton produces significant impact in lakes because of strong, cascading trophic effects (Brooks & Dodson, 1965; Jeppesen *et al.*, 1997a). In temperate shallow lakes lacking a

hypolimnetic refuge (which favours vertical migration), chemical cues from potential predators lead the large-bodied zooplankton to perform diel horizontal migration (DHM; Burks *et al.*, 2002). The maintenance of a herbivorous zooplankton population that can graze algae has individual, population and even ecosystem consequences (Brönmark & Hansson, 2000; Jeppesen *et al.*, 2002).

In shallow temperate lakes, submerged plant beds provide a daytime refuge for the pelagic zooplankton, which often move to feed in open water at night, when the predation risk from fish is lower (Timms & Moss, 1984; Burks *et al.*, 2002). Both field (Pennak, 1966) and laboratory studies (Pennak, 1973) have shown that plants are avoided by zooplankton, but that the presence of predators induces zooplankters to overcome their repellence and to seek refuge in the vegetation (Lauridsen & Buenk, 1996; Lauridsen & Lodge, 1996). This effect of macrophytes on the interactions between zooplankton and planktivorous fish constitutes an important mechanism by which submerged plants maintain clear water in shallow temperate lakes (Scheffer *et al.*, 1993; Lauridsen *et al.*, 1996). The refuge effect for *Daphnia*, however, varies with the trophic state of the lake (Lauridsen *et al.*, 1999), the structure of the fish community (Jeppesen *et al.*, 1997a) and with several plant characteristics, such as species, architecture, bed size and per cent of the lake volume inhabited (Schriver *et al.*, 1995; Lauridsen *et al.*, 1996). Previous work suggests that at both extremes of the nutrient gradient, submerged plants offer little refuge for zooplankton (Jeppesen *et al.*, 1997b). In low-nutrient lakes, clear water and the scarcity of macrophytes enhance fish predation on zooplankton whereas, under hypertrophic conditions, the refuge effect is weak because of the scarcity or absence of submerged plants and often high densities of planktivorous fish (Jeppesen *et al.*, 1997b). Besides the submerged and floating-leaved plants, other growth forms, such as emergent plants, may be important for the structure and migration patterns of zooplankton (Nurminen & Horppila, 2002; R.L. Burks, H. Michels, M.A. González-Sagrario & E. Jeppesen, unpublished data).

Studies on DHM have so far mainly focused on northern, temperate lakes (Burks *et al.*, 2002), while macrophyte-fish-zooplankton interactions in tropical and subtropical systems remain to be elucidated in more detail (but see Jeppesen *et al.*, 2005; Iglesias *et al.*,

in press; Jeppesen *et al.*, in press, a). Such interactions may be more complex in tropical and subtropical lakes (Lazzaro, 1997). Fish zooplanktivory is expected to be stronger because of (i) multiple or continuous reproductive events by fish (Paugy & Lévêque, 1999), (ii) low densities of large, specialist piscivores frequently exhibiting sit-and-wait hunting behaviour (Quirós, 1998), (iii) widespread omnivory (Branco *et al.*, 1997; Yafe *et al.*, 2002) and (iv) high population densities of small and juvenile fish (Mazzeo *et al.*, 2003). Although the necessity of a daytime refuge for zooplankton appears strong in subtropical and tropical lakes, DHM is not so obviously advantageous, because small and juvenile fish are often numerous within macrophyte beds in these lakes (Conrow, Zale & Gregory, 1990; Agostinho, Gomes & Ferreira, 2003; Meerhoff *et al.*, 2003). However, all macrophyte growth forms (emergent, submerged, floating-leaved and large free-floating) can be extremely dense in subtropical and tropical lakes (Talling & Lemoalle, 1998) and thus might potentially provide refuge for the zooplankton against predation despite high fish density. Contrary to temperate lakes, (sub)tropical lakes under eutrophic and hypertrophic conditions may contain a high density of large free-floating plants, which may offer a potential refuge for zooplankton when submerged plants are scarce.

Using laboratory 'habitat choice' experiments, we determined the effects of three free-floating plants, *Eichhornia crassipes* Mart. (Solms) (water hyacinth), *Pistia stratiotes* L. (water lettuce) and *Salvinia auriculata* Aubl (water fern) and the submerged plant *Ceratophyllum demersum* L. (coontail or common horntwort), on horizontal movement by the water flea *Daphnia obtusa* Kurz. We performed these experiments in the absence and presence of alarm signals from crushed conspecifics and chemical stimuli from the planktivorous fish *Cnesterodon decemmaculatus* Jenyns. The free-floating plant species are native to (sub)tropical South America and so is *C. decemmaculatus*, present in Argentina, Uruguay and the south of Brazil (Rosa & Costa, 1993). *Ceratophyllum demersum* is cosmopolitan and widely distributed in subtropical lakes. Based on data from temperate lakes, we would expect daphniids to avoid (i.e. swim away from) the plants in the absence of predation risk or alarm signals, but to seek refuge in the plants when facing risk of predation. However, if predation risk is actually higher in plant beds in (sub)tropical systems, an alternative outcome

might be that *Daphnia* would persistently avoid plants.

Methods

Experimental design

We collected *D. obtusa* from Lake Rivera (34°55'S, 56°10'W Montevideo, Uruguay), from patches of open water near the shore. Lake Rivera is a hypertrophic lake with large fluctuations in the density of the omnivorous-planktivorous fish *C. decemmaculatus* (N. Mazzeo, F. Scasso & J. Garcia, unpublished data) and is often covered by free-floating plants (mainly *E. crassipes*) although no submerged plants have been recorded. To be sure of obtaining predator-inducible clones, we cultured the individuals collected in both free-floating and submerged vegetation and allowed *C. decemmaculatus* to feed on the daphniids for about 2 months before the experiments. We used 1.5–2.0-mm egg-carrying daphniids, pre-incubated in the laboratory in 16 h:8 h L : D light chambers, at a constant temperature of 20 °C and fed in excess on dried yeast (daphniids were whitish as a result). We collected the plants from different lakes and thoroughly rinsed them several times in the laboratory to remove any attached organism. We maintained the plants under the natural photoperiod, adding a nutrient-rich solution until the start of the experiments. The fish (adult *C. decemmaculatus*, about 2.5–3.0 cm length) came from a *Daphnia*-free hypertrophic lake. We kept them in aerated aquaria and fed them according to two feeding regimes: half received

Daphnia and the other half dried *Tubifex* sp., the latter avoiding chemical cues from crushed *Daphnia*.

Each experimental unit consisted of two transparent cylindrical tanks (8-L volume each) connected at the bottom by a 1-m long glass tube with a diameter of 5 cm. The water flow within the set-up permitted the generation of a gradient in the chemical cues (which we confirmed by a dye-study following the same experimental protocol). We used two units at a time, filled with 10-L of spring water, thus avoiding any potential cues prior to the experiments. One unit received the treatment and the other the spring water as a control. The respective treatment was applied in one side of the unit, chosen at random, 30 min before the addition of daphniids, to build up a chemical cue gradient. We then gently added ten large daphniids through a vertical tube (2.0-cm diameter) exactly at the centre of the connecting tube. The treatments (13 in total, Table 1) included: the four plant species covering the whole area of one tank; fish on the two feeding regimes (in a mesh cage in the water but without direct contact with the daphniids); 40 crushed adult *Daphnia* ('alarm signals' *sensu* Pijanowska, 1997); and double treatments: each plant species again in one side and crushed *Daphnia* in the opposite side of the experimental unit. Moreover, to determine whether the behaviour of the daphniids towards the free-floating plants was either chemically or mechanically induced, we used, respectively, suspended filter-paper bags with roots of *E. crassipes* (thus removing the shade effect of an intact plant) and plastic structures mimicking the architecture of the root system and the shade produced by the leaves of

Table 1 Summary of the experiments performed in the study, indicating each treatment and the respective hypothesis to be tested and the methodology employed

No.	Short name	Hypothesis	Method
1	<i>E. crassipes</i>	Daphniids avoid plants	Water hyacinth covering one tank
2	<i>P. stratiotes</i>	Daphniids avoid plants	Water lettuce covering one tank
3	<i>S. auriculata</i>	Daphniids avoid plants	Water fern covering one tank
4	<i>C. demersum</i>	Daphniids avoid plants	Coontail covering one tank
5	Roots	Plant avoidance is chemically induced	Suspended roots of <i>E. crassipes</i>
6	Plastic	Plant avoidance is mechanically induced	Plastic mimic of <i>E. crassipes</i>
7	Fish fed <i>Tubifex</i>	Daphniids avoid potential predators	Three caged fish in one tank
8	Fish fed <i>Daphnia</i>	Daphniids avoid 'latent' alarm signals	Three caged fish in one tank
9	Alarm signals	Daphniids avoid alarm signals	40 crushed daphniids in one tank
10	Refuge <i>E. crassipes</i>	Daphniids seek refuge in the plants	Combination exp 1 + 9
11	Refuge <i>P. stratiotes</i>	Daphniids seek refuge in the plants	Combination exp 2 + 9
12	Refuge <i>S. auriculata</i>	Daphniids seek refuge in the plants	Combination exp 3 + 9
13	Refuge <i>C. demersum</i>	Daphniids seek refuge in the plants	Combination exp 4 + 9

E. crassipes. We did not include leaves in the 'chemical effect' experiment as only a minor part is normally in contact with the water and therefore the chemical cues coming from the root system are more likely to promote the response of *Daphnia*.

After allowing for 15-min acclimation, we recorded the position of individual water fleas at 15, 30, 60 and 90 min after their introduction as being: (i) in the tank without the treatment (interpreted as repellence), (ii) in the tank with the treatment (attraction) or, if in the connecting tube, (iii) repellence if the daphniids were in the half of the tube furthest from the treatment, or attraction if they were in the half closest to the treatment. The same procedure was applied in the control unit (spring water in both tanks) located besides the treatment unit. The experiments took place in a windowless room with a uniform light regime over the units. We replicated each experiment five times over a short period, always using different animals and plants and rinsing the units thoroughly with distilled water before the next experiment. All other conditions, ambient light, and air and water temperatures remained constant.

Statistical analyses

The proportion of daphniids (p) showing repellence (i.e. proportion in the tank opposite to the treatment + corresponding half side of the tube) required transformation [$\arccosine \sqrt{p}$] prior to the statistical analyses (Sokal & Rohlf, 1997). To compare the short and long-term responses to each treatment, we analysed each experiment with paired t -tests (treatment *versus* control, considering the proportions of *Daphnia* in the same side of the units) at 15 and 90 min. We acknowledge that these measurements do not represent independent data; however, we wanted to compare the speed of the response beside the long-term one. The consequent relatively large number of tests increases the overall probability of making type I errors. Rather than applying a conservative correction (e.g. Bonferroni), we chose to present the true P -values of the tests. Although some of the low P -values found could be due to type I errors, the pattern of significances in the tests was consistent and pointed in the same direction within the experiments (whereas type I errors should occur randomly and not in patterns). Moreover, in most cases we found P -values even lower than the alpha we should apply

based on Bonferroni corrections (e.g. in the first set of experiments the Bonferroni-corrected P -value would be $P = 0.0063$, as we performed eight paired t -tests).

We tested the differences between particular sets of experiments with one- or two-way repeated measures ANOVA (followed by Tukey HSD if significant differences appeared), after subtracting the transformed proportion of daphniids in each control from the respective treatment. As the RMANOVA requires no temporal trend in the data we excluded the 15 min measurements (see *E. crassipes* in Fig. 1 as example of the temporal pattern in some of the experiments).

Results

Under control conditions, the distribution of daphniids in the experimental unit changed slowly and showed a relatively homogeneous distribution after 30–60 min of the experiments.

Daphnia response to free-floating and submerged plants

In the absence of other stimuli, *D. obtusa* significantly avoided both free-floating and the submerged plants. We found significant differences among the four plant species (one-way RMANOVA $F_{3,16} = 4.84$, $P = 0.0139$), with the repellence by *E. crassipes* being the strongest and that to the submerged plant being the weakest (Fig. 1).

The repellence by *E. crassipes* occurred quickly in our experiment. After 15 min, $90\% \pm 10\%$ (SD) had swum towards the opposite side ($t_{0.05(4)} = 4.94$, $P = 0.007$). After 30 min and until the end of the experiment, all daphniids had moved away from the plants (90 min: $t_{0.05(4)} = 20.05$, $P < 0.0001$).

A rapid strong repellence also occurred with *P. stratiotes*. After 15 min, $68\% \pm 26.7\%$ (SD) of the daphniids were located opposite to the plants, while $22\% \pm 17.9\%$ were in the plant side of the connecting tube ($t_{0.05(4)} = 4.07$, $P = 0.0151$). At the end of the experiment, $96\% \pm 5.5\%$ were inside the opposite tank ($t_{0.05(4)} = 8.81$, $P = 0.0009$).

The response to the presence of *S. auriculata* occurred quickly and was stronger than that of *P. stratiotes* (Fig. 1). After 15 min, on average $96\% \pm 8.9\%$ of the daphniids displayed repellence by the plants ($t_{0.05(4)} = 6.6$, $P = 0.0028$), while after 90 min, all had swum away from the plants ($t_{0.05(4)} = 15$, $P = 0.0001$).

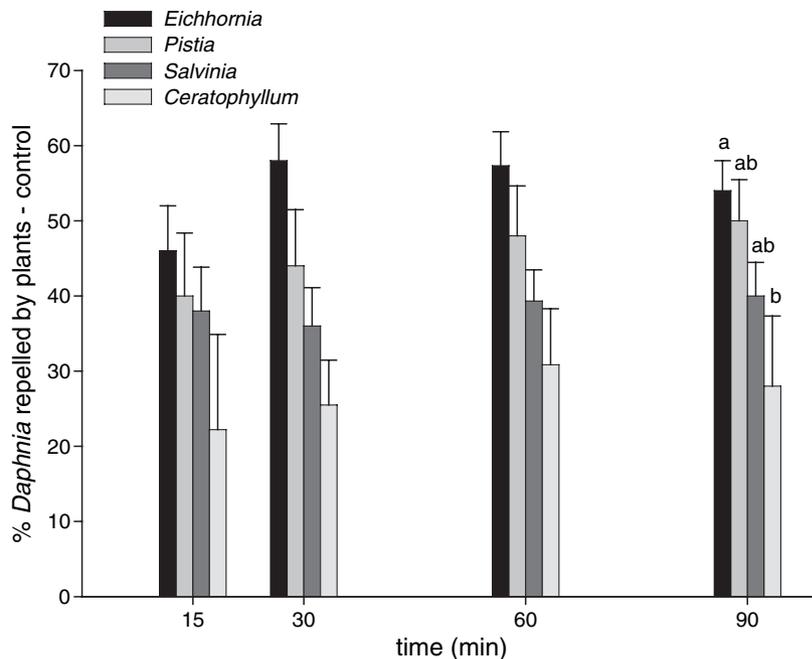


Fig. 1 Net effect of the plants *E. crassipes*, *P. stratiotes*, *S. auriculata* and *C. demersum* on the horizontal movement of *D. obtusa*. Data represent the difference between the proportion of *Daphnia* repelled by the plants and the proportion in the respective side of the control unit (mean \pm 1 SE). The weaker the repellent effect of the plant, the closer to 0% is the difference. Small letters over 90-min bars indicate significant differences among treatments for the transformed data, according to RMANOVA and Tukey HSD tests.

In the case of submerged *C. demersum*, the repellence occurred more slowly and was slightly weaker than with the previous plant species. After 15 min, 87% \pm 19% (SD) had swum towards the opposite side ($t_{0.05(4)} = 2.14$, $P = 0.0986$). In the long run, most daphniids moved away from the plant (90 min: $t_{0.05(4)} = 3.47$, $P = 0.0254$).

We performed further experiments to elucidate whether the repellence by the free-floating plants was

either chemically or mechanically induced, using *E. crassipes* as prototype. We found that both the chemical cues from the roots (90 min: $t_{0.05(4)} = 6.8$, $P = 0.0024$) and the mechanical structure (90 min: $t_{0.05(4)} = 4.97$, $P = 0.0076$) promoted *Daphnia* avoidance. Although the degree of the response to both treatments was similar, daphniids responded more quickly to real roots than to plastic plants (Fig. 2). We found a significant avoidance of roots after 15 min,

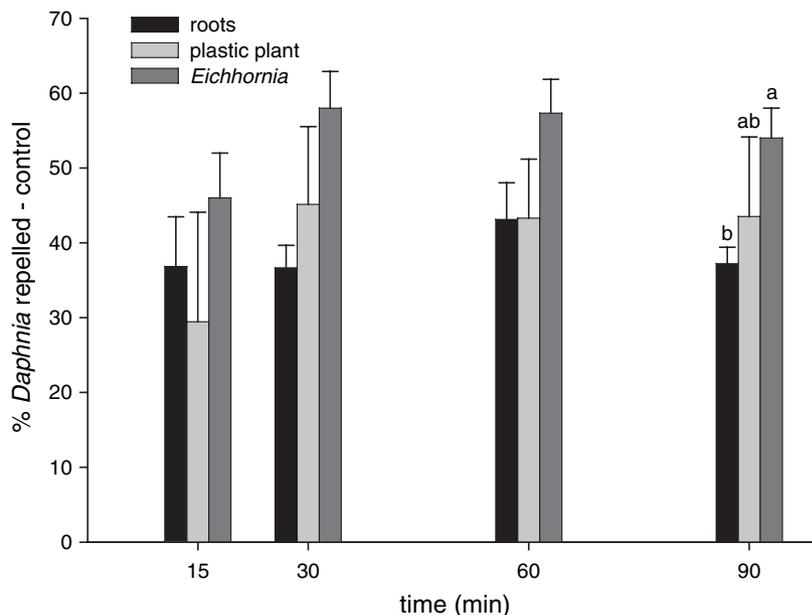


Fig. 2 Horizontal movement of *D. obtusa* in the presence of water hyacinth (*E. crassipes*), water hyacinth roots, and plastic structures mimicking the root system and the shade by the leaves. Data represent the difference between the percentage of *Daphnia* repelled by each treatment and the percentage in the respective side of the control unit (mean \pm 1 SE). Small letters over 90-min bars indicate significant differences among treatments for the transformed data, according to RMANOVA and Tukey HSD tests.

but of plastic plants. According to the RMANOVA, complete plants repelled daphniids significantly more than the roots alone ($F_{2,12} = 4.65$, $P = 0.0321$), whereas the effects of the plastic plants did not differ significantly from the other two treatments (Tukey *post hoc* test, Fig. 2).

Daphnia response to fish

Although most daphniids moved to the side with the lowest concentration of fish cues (i.e. the tank opposite to the one with the fish) in several of the replicated experiments, the presence of *C. decemmaculatus* did not significantly affect the behaviour of *D. obtusa*. Most daphniids displayed an almost homogeneous distribution along the unit. The same behaviour occurred for fish fed with *Tubifex* (15 min: $t_{0.05(4)} = 1.08$, $P = 0.3405$; 90 min: $t_{0.05(4)} = 0.81$, $P = 0.4626$) as for those fed with *D. obtusa* ($t_{0.05(4)} = -0.62$, $P = 0.2499$; $t_{0.05(4)} = -0.67$, $P = 0.2991$, after 15 and 90 min, respectively). This means that neither *C. decemmaculatus* itself (fish fed on *Tubifex*) nor fish fed on *Daphnia* (which could be associated with some *Daphnia* alarm substance) repelled *D. obtusa*.

Daphnia response to 'alarm signals'

Although daphniids did not swim away from fish, the chemical cues coming from crushed conspecifics repelled them, although not immediately (Fig. 3).

Initially, the distribution was relatively homogeneous (15 min: $t_{0.05(4)} = 1.27$, $P = 0.2722$), but after 30 min more than 40% of the daphniids had moved to the opposite side, while 13% remained inside the tank with the cues. After 90 min, $70.4\% \pm 10.2\%$ showed repellence while only $25.3\% \pm 11.2\%$ had moved to the side with the cues ($t_{0.05(4)} = 3.76$, $P = 0.0197$). On the basis of these results, we decided to use crushed daphniids rather than *C. decemmaculatus* cues to trigger refuge search.

Plants as a refuge for *Daphnia*

To test the hypothesis that *D. obtusa* would overcome their original avoidance of plants when faced with the risk of predation or alarm signals, we placed each of our four experimental plant species and 40 crushed daphniids at opposite sides of the experimental unit. Contrary to our primary expectations, daphniids did not move to the plants in the presence of crushed conspecifics, regardless of the plant species and growth form (Fig. 4).

However, the degree and speed of the response differed with plant species. In the case of *E. crassipes*, 15 min after introduction most daphniids moved towards the side with the crushed conspecifics ($t_{0.05(4)} = -6.36$, $P = 0.0031$) and after 30 min all had swum away from the plants (90 min, $t_{0.05(4)} = -11.2$, $P = 0.0036$). In contrast, in the experiment with *P. stratiotes*, no significant differences occurred after

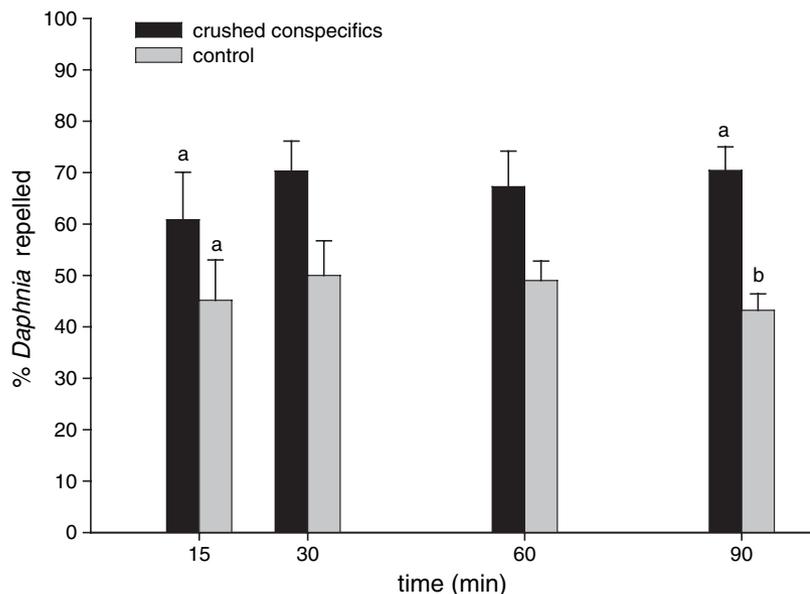


Fig. 3 Response of *D. obtusa* exposed to alarm signals (crushed conspecifics). Data represent the percentage of *Daphnia* repelled by the crushed daphniids (black) compared with the respective side of the control unit (grey) (mean \pm 1 SE). Small letters over bars indicate results from paired *t*-tests after transforming the data.

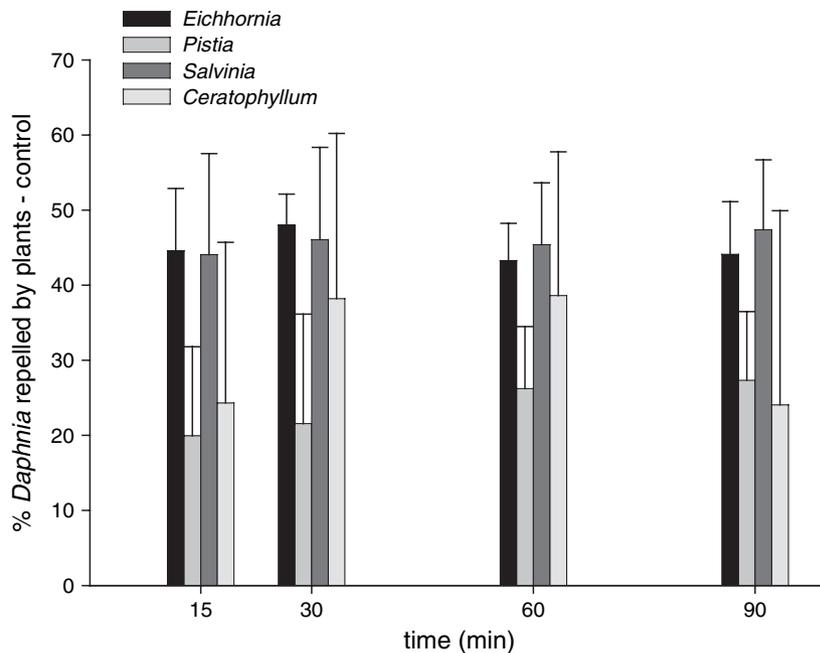


Fig. 4 Choice of *D. obtusa* when exposed simultaneously and on opposite sides of the unit, to crushed conspecifics and *E. crassipes*, *P. stratiotes*, *S. auriculata*, and *C. demersum*. Data show the net repellent effect of the plants, i.e. the difference between the proportions of *Daphnia* repelled by the plants and the proportion in the respective side of the control unit (mean \pm 1 SE). If plants were attracting the daphniids ('refuge effect'), the percentage differences would be negative. Treatments did not differ significantly.

15 min compared with the control ($t_{0.05(4)} = -1.95$, $P = 0.122$). With time, instead of searching for the plants, most daphniids ($88\% \pm 4.2\%$) moved towards the side with crushed *Daphnia* cues (90 min, $t_{0.05(4)} = -4.13$, $P = 0.014$). Also, as with *S. auriculata*, more daphniids moved towards the crushed conspecifics rather than to the plants ($t_{0.05(4)} = -4.8$, $P = 0.009$ and $t_{0.05(4)} = -7.8$, $P = 0.001$; for 15 and 90 min, respectively).

In contrast, in the case of the submerged species, we detected no daphnid preferences (i.e. equal repellence response to both stimuli), as there were no significant differences between the treatment and the control unit, neither at the beginning (15 min, $t_{0.05(4)} = 0.86$, $P = 0.437$) nor at the end of the experiment (90 min, $t_{0.05(4)} = 0.11$, $P = 0.3283$). The variation among the replicates in the presence of crushed conspecifics was much larger than in the presence of the plant *C. demersum* alone.

In most cases, daphnid repellence by the plants was weaker when daphniids were also exposed to crushed conspecifics than when exposed to plants alone (compare Fig. 4 versus Fig. 1). This weaker repellence was more noticeable in the case of *P. stratiotes*. We again compared the repellence by the plants under the scenario of the two simultaneous stimuli and found that the degree of repellence did not then differ significantly between the four plant species (one-way RMANOVA $F_{3,16} = 1.55$, $P = 0.2412$; Fig. 4). Such dif-

ferences to the response to the plants (Fig. 4 versus Fig. 1), indicate interaction between the factors 'plants species' and 'alarm signals'. We tested for this but found no significant interaction between these factors (two-way RMANOVA $F_{3,32} = 1.17$, $P = 0.3349$).

Discussion

Daphnia obtusa clearly avoided the selected set of plants that are common in (sub)tropical lakes, despite cues to the risk of predation from a very common planktivorous fish and from crushed conspecifics. This is in contrast to the findings for submerged plants in choice experiments (Lauridsen & Lodge, 1996) and field studies (Lauridsen & Buenk, 1996) conducted in north temperate systems. Although the repellence by plants weakened in the presence of alarm signals, our results suggest that the costs of exposure to the plants and particularly to the free-floating species, do not outweigh the benefits of swimming away from predators or alarm signals. These results are more consistent with our alternative hypothesis, that *Daphnia* would avoid the plants, perhaps because of a permanently higher predation risk here.

The behaviour of *Daphnia* in these 'habitat choice' experiments followed our expectations when faced with the plants alone and with crushed conspecifics, but not in the case of the omnivore-planktivore *C. decemmaculatus*. We had expected the fish would

repel *Daphnia*, as has been found in studies with several temperate fish species subjected to different food regimes (Stirling, 1995; Von Elert & Pohnert, 2000). *Daphnia* even avoided non-native predators, as long as the predators had previously fed on *Daphnia* (Stabell, Ogebebo & Primicerio, 2003). Moreover, it has been demonstrated that even some *Daphnia* clones coming from fishless habitats can respond to fish kairomones (Boersma, Spaak & De Meester, 1998; Michels & De Meester, 2004).

The fish community of Lake Rivera, where the daphniids were collected, has changed extensively and fish density has ranged from very high to very low (N. Mazzeo & M. Meerhoff, unpublished data). We therefore expected our experimental *Daphnia* population to respond to fish cues. Despite the 2-month exposure of a daphniid population to several cues prior to the experiments, the individuals used in our experiments could potentially have been clones not responsive to fish with behavioural traits. Such interclonal differences have been described for *D. magna* (De Meester, 1993). Alternatively, the short period of our experiments (45 min before recording first daphniid position, 120 min in total) may have been insufficient to generate a high enough concentration of fish cues. However, the response of *D. obtusa* to alarm signals indicates the recognition of a non-specific predation risk (Pijanowska, 1997) and the non-use of the plants as a refuge was evident. This response persisted even when the number of crushed conspecifics was raised from 40 to 100 (M. Meerhoff, C. Fosalba & C. Bruzzone, unpublished data).

Gliwicz & Rykowska (1992) suggested that high predation risk in the littoral zone could be the reason that zooplankton in temperate lakes sometimes avoid the shoreline. Several studies in the subtropics have shown high densities of fish within plant mats, both submerged and free-floating. Stands of submerged and free-floating plants act as important nursery areas for juvenile fish of many species in several subtropical lakes (in Florida, U.S.A., Conrow *et al.*, 1990; Uruguay, Meerhoff *et al.*, 2003). The aversion to plants shown in our experiments may not be elicited by the plants themselves, but rather by the characteristically high densities of zooplanktivorous fish seeking refuge or feeding within the vegetation in subtropical lakes. *Daphnia* may use compounds released by macrophytes as a chemical signal (*sensu* Larsson & Dodson, 1993) of a continuous and higher predation risk

among plant beds than in the pelagic where the fish density is lower. Also, higher invertebrate predation (Burks, Jeppesen & Lodge, 2001a,b; Van de Meutter, Stoks & De Meester, 2004, 2005) may potentially enhance the effects of such high densities of fish, although evidence is scarce about invertebrate predator abundance and their interaction with fish and zooplankton in the various types of plant communities in lakes in the (sub)tropics (but see González-Sagrario & Balseiro, 2003).

Our results, although obtained from small-scale simple 'habitat choice' (one or two chemical cues at a time) experiments, suggest that plants of both life-forms may signal danger rather than potential refuge for daphniids in the subtropics, but that free-floating plants did so to a larger extent than submerged plants. These results concur with field studies where lower densities of crustacean zooplankton occurred under free-floating plants than within submerged plants (Meerhoff *et al.*, 2003 and M. Meerhoff, C. Iglesias & J. Clemente, unpublished data from several lakes). Brendonck *et al.* (2003) also found a lower abundance of zooplankton under mats of *E. crassipes* than in open water in a subtropical lake in Zimbabwe. A differential response of *Daphnia* to the free-floating (strong avoidance) than to the submerged plants (weak avoidance) could be due to several factors. The presence of either free-floating or submerged plants might have different optical consequences, which may be used by daphniids as a proximate cause of migration (as suggested by Siebeck, 1980 and discussed by Ringelberg & Van Gool, 2003 for DVM). Rejection by daphniids of the free-floating plants in our experiments seemed to be mediated both visually and chemically. The relative importance of both mechanisms in a natural environment probably varies with the species involved. The dense structure of *E. crassipes* and *P. stratiotes* roots may hamper movement by daphniids (as suggested for submerged plants by Pennak, 1973). Chemical aversion was more conspicuous in the case of *S. auriculata*, probably because its roots are very small and not easily visible in our experimental set-up. A direct negative chemical effect may occur if some of the chemical compounds released by these plants affected the reproduction or development of *Daphnia*, as Burks, Jeppesen & Lodge (2000) found for the submerged plant *Elodea canadensis* Michx. *Eichhornia crassipes* and the also floating *Stratiotes aloides* L., affect negatively the alga *Scenedesmus*

(Sharma, Gupta & Singhal, 1996; Mulderij, Mooij & Van Donk, 2005), but further studies are needed to elucidate if free-floating plants can also affect *Daphnia* negatively. Lower food quality (because of the shading of phytoplankton) and particularly, detrimental physico-chemical conditions under dense floating mats (such as persistently low oxygen concentration compared to that within beds of submerged plants: Rodríguez-Gallego *et al.*, 2004), may be the complementary causes of the aversion towards this type of plants.

Our results have important implications for understanding the habitat choice and migration patterns of *Daphnia* and probably other cladocerans in shallow (sub)tropical lakes and are supported by field evidence. In subtropical shallow Lake Blanca (Uruguay), the refuge capacity of the submerged plants for zooplankton was weak and changed strongly with seasons and other predator avoidance strategies besides DHM (e.g. vertical migration) seemed to occur (Iglesias *et al.*, in press).

Experimental and field observations in temperate systems have shown strong refuge-mediated cascading effects of submerged plants on the entire ecosystem and on water clarity (Søndergaard & Moss, 1997; Jeppesen *et al.*, 2002). Free-floating and probably also submerged macrophytes (as they seem not to provide a refuge) may therefore have no such positive effect on water transparency in subtropical and tropical lakes. This agrees well with empirical data from numerous Florida lakes showing minor effect of high submerged plant densities on water clarity (Bachmann *et al.*, 2002; Jeppesen *et al.*, in press, b) compared with results from lakes in the north temperate zone (Moss, 1990; Scheffer *et al.*, 1993). However, more experimental and field studies are needed, involving more plant species, other key structuring cladocerans and multiple cues, to elucidate fully the role of macrophytes in warm lakes and their potential cascading effects on water clarity.

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