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Littoral zone structures as *Daphnia* refugia against fish predators

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Abstract

The assumption that macrophytes can provide zooplankton a daytime refuge against fish predation is central to the diel horizontal migration hypothesis. However, previous observations and experiments have shown that large-bodied zooplankton avoid macrophytes. To directly test these contrasting roles of macrophytes, we measured the reaction of *Daphnia pulex* to macrophytes (*Elodea canadensis* or plastic) in the presence and absence of chemical cues from two commonly occurring European fishes, roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*). In the same series of laboratory experiments, we then tested the ability of different densities of *E. canadensis* and plastic macrophytes to reduce predation by roach or perch. In the absence of fish, only 27% of daphnids occurred in the macrophytes. However, 70–80% of daphnids occurred in the macrophytes when either roach or perch occurred. In addition, significantly more daphnids occurred in plastic versus real macrophytes, indicating that some chemically mediated avoidance of real macrophytes by *Daphnia* occurred. In the presence of fish chemical cues from either roach or perch, the differences between real and plastic macrophytes quickly disappeared as daphnids moved into macrophytes. However, this behavior decreased mortality only from roach predation, because perch spent significantly more time than roach foraging among macrophytes. Thus, although daphnids sought macrophyte refuge in the presence of both fishes, the effectiveness of the refuge depended on macrophyte density and predator identity. These results support the idea that macrophytes can increase survival of *Daphnia* populations in shallow lakes, depending on the fish species present.

Daphnia, the large-bodied zooplankters whose herbivory on phytoplankton is so important in trophic cascades (Carpenter et al. 1985; Jeppesen 1998), are typically thought of as purely pelagic. Consistent with this notion, “shore avoidance” (sensu Hutchinson 1967) by *Daphnia* has been documented (Boikova 1986; Lauridsen et al. 1999). Also, *Daphnia* often use deep waters to migrate vertically and to hide from predators during the day in stratified lakes (Lampert 1993). However, this predator-avoidance strategy of diel vertical migration is likely less advantageous in shallow lakes, where light may penetrate to the lake bottom and where

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thermal stratification may not exist. In these systems, *Daphnia* sometimes use diel horizontal migration (DHM) to seek daytime refuge from predation among structure in littoral zones (Timms and Moss 1984; Lauridsen and Buenk 1996; Stansfield et al. 1997; Lauridsen et al. 1998; Moss et al. 1998).

Understanding this interaction between macrophytes and *Daphnia* is critical to better management—including bio-manipulation—in shallow (unstratified) lakes (Perrow et al. 1997; Jeppesen 1998), which are the most common lakes in the world (Wetzel 1990). Shallow lakes differ from the better-studied deep lakes in ways that may influence the *Daphnia*–littoral zone relationship (Moss et al. 1997). Biomass of fishes per unit volume is higher in shallow lakes than in deep lakes, and high densities of fishes can sustain predation pressure on zooplankton by relying on alternative benthic food sources (Blumenshine et al. 1997; Jeppesen et al. 1998). Thus, predation pressure on daphnids is potentially higher during the day in shallow versus deep lakes (Jeppesen 1998; Jeppesen et al. 1998), therefore increasing the importance of daytime refuge for *Daphnia*.

However, the occurrence of DHM by large-bodied zooplankton apparently contradicts documented avoidance of macrophytes by *Daphnia* (Hasler and Jones 1949; Pennak 1973; Dorgelo and Heykoop 1985). This paradox has been only partially addressed in previous work. Daphnid responses to both fishes and macrophytes are partially chemically

mediated. For example, in the presence of green sunfish (*Lepomis cyanellus*), daphnids overcame their previous aversion to macrophytes (Lauridsen and Lodge 1996). Yet it is not clear whether daphnids respond similarly to chemicals from other fish species, nor is it clear how the structural type of macrophyte influences this behavioral response.

Laboratory studies have tested the manner by which imitation submerged and floating macrophytes affected the foraging abilities of planktivorous fishes (Winfield 1986; Diehl 1988). For example, the number of chironomid larvae captured by bream (*Abramis brama*), roach (*Rutilus rutilus*), and perch (*Perca fluviatilis*) decreased with increasing density and complexity of artificial structure (Diehl 1988). However, perch were less affected by structural density than bream or roach (Diehl 1988), and perch consumed more *Daphnia* amid complex habitat than either roach or rudd (*Scardinius erythrophthalmus*) in a separate study (Winfield 1986). Nevertheless, field studies show that high petiole densities of the water lily, *Nuphar lutea*, allowed *Daphnia* to persist despite the presence of young perch (Moss et al. 1998). Yet it is still unclear to what extent accurate imitations of macrophytes or real submerged macrophytes decrease *Daphnia* mortality due to predation by various fishes.

The objective of this study involved testing whether the movement of daphnids into macrophytes reduced their mortality from fish predation. In a series of laboratory experiments, we examined how *Daphnia* responded to the presence of real and plastic macrophytes before and during exposure to chemical cues from two planktivorous fishes, either young roach or young European perch. We expected daphnids to avoid macrophytes in the absence of fish but to move into macrophytes in the presence of fish chemical cues. We also expected daphnids to use macrophyte habitat more when roach were present, relative to when perch were present. We anticipated this result because roach typically avoid structure in the absence of piscivores (Persson 1993), whereas European perch are efficient foragers in vegetation (Winfield 1986). Furthermore, we tested the ability of different densities of *Elodea canadensis* and plastic macrophytes to reduce *Daphnia* mortality from predation by these two European planktivores. We predicted that high densities of refuge would provide the best protection for *Daphnia*, although we expected that perch would forage more effectively than roach.

Methods

Behavioral response of Daphnia to fish and macrophytes—For our laboratory experiments, we collected *Daphnia pulex* from Lake Engelsholm, Denmark, in early August and maintained daphnids in a laboratory culture fed daily with *Scenedesmus acutus* in excess of 1 mg carbon L⁻¹ for 6 weeks before we conducted the experiments. We studied habitat choice (open water or macrophytes) of *D. pulex* in cylindrical tanks (65 liter, 50-cm diameter, 33-cm height) in three experiments: (1) without fish cues, (2) with chemical cues from roach, or (3) with chemical cues from European perch. Real or plastic macrophytes (as described in Lauridsen and Lodge 1996) occurred within an inner area (30-cm

diameter), for which macrophyte density was expressed as percent plant volume infested (%PVI). PVI is calculated as the area of macrophyte coverage multiplied by the plant height divided by the water depth (Canfield et al. 1984). A mesh cylinder (4-mm² mesh) demarcated this vegetated area from an outer ring of open water (1.8:1 open water:macrophyte volume). The position of the macrophytes and open water remained the same for each treatment in all three experiments. *Daphnia* could freely move between the inner vegetated and the outer open water areas (Burks pers. obs.).

Each experiment included one tank without macrophytes (0% PVI), three tanks with plastic macrophytes (28, 62, and 94% PVI), and three tanks with *E. canadensis* (waterweed) (22, 44, and 70% PVI). These seven treatments were repeated over time for replication. We grouped stems of real and plastic macrophytes into five bunches of five stems/bunch and secured the stems with silicone sealant to the bottom of the tank. Different plant heights (11, 21, and 28 cm) were used to construct low, medium, and high PVI, respectively. The shoots of the plastic macrophytes (imitation *Myriophyllum*) had a larger diameter than did those of the *E. canadensis*, resulting in slightly higher PVI values for plastic versus real macrophytes at each density.

Ideally we would have conducted the three experiments simultaneously so that they would have better constituted one experiment. However, we were unable to obtain roach and perch simultaneously, and because of the sensitive nature of these small fishes, we had to conduct experiments with them quickly. We conducted one replicate per day starting with experiments including roach (21–23 September 1998; *N* = 3); then we conducted experiments in the absence of fish (27–29 September 1998; *N* = 3); finally, we conducted experiments with perch (29 September–1 October 1998, 8 October 1998; *N* = 4). Therefore, we completed all three experiments within 2½ weeks. All replicates took place in climate-controlled rooms (20–22°C) during daylight hours (16:8 light:dark). One individual made all of the observations. Individual daphnids and fish were only used once. All experiments used tap water to eliminate the possibility of preexisting macrophyte or fish chemical cues, and tanks were washed and refilled with fresh water for each replicate. We also used freshly collected *E. canadensis* for each replicate. Thus, given the short time between experiments and the identical lab conditions for all three experiments, the potential for time to confound comparisons across experiments is very low. Therefore, we statistically compared results between experiments.

In the experiment without fish cue, we observed the position (open water or macrophytes) of 20 large (>2-mm) *D. pulex* in each of our seven treatments. At the beginning of the experiments, daphnids were placed in the center of the tanks (where, if present, macrophytes occurred). We observed daphnid position 15, 30, 45, and 60 min after release. We recorded the number of daphnids that occurred in the macrophytes. All daphnids were sufficiently large and possessed sufficient coloration to facilitate easy location, thus allowing our observations to be 100% efficient. In the other two experiments, we again observed the position of 20 large *D. pulex* in the seven tanks at the same time intervals. However, in these experiments, we gently lowered a fish into a

Table 1. Repeated measures ANOVA (rmANOVA) results for two preliminary analyses of daphnid behavior. The percentage of daphnids that occurred in macrophytes is the repeated measure over time (15, 30, 45, or 60 min). (A) One-way rmANOVA (between subject effects) testing if fish cue (present or absent) influenced daphnid behavior over time in the absence of macrophytes; (B) Two-way rmANOVA (between subject effects) testing if macrophyte density (PVI: low, medium, or high) or macrophyte type (real or plastic) influenced daphnid behavior over time in the absence of fish.

Effect	Df	MS	F	P
(A)				
Fish cue	2	29.635	0.440	0.661
Error	7	67.336	—	—
(B)				
Macrophyte density	2	10.764	0.108	0.898
Macrophyte type	1	1,168.056	11.762	0.005
Macrophyte density × macrophyte type	2	192.014	1.934	0.187
Error	12	99.306	—	—

PVI, plant volume infested.

small cylindrical cage (1-mm² mesh, 22-cm height, 10-cm diameter) after the 30-min observation. As a control, this cage occurred in all treatments in all experiments and was present before the daphnids were added into the tanks. We included a 30-min prefish period as an additional control because the experiment without fish was not conducted simultaneously. In experiments with fish, a single, 1+ roach (mean length ± SD, 84 ± 5 mm; mean weight ± SD, 10.5 ± 1 g) or 0+ European perch (length: 46 ± 4 mm; weight: 1.5 ± 0.3 g) was held in the Nitex cage, which we placed in the outer ring of open water.

Statistical analyses—To understand fish–*Daphnia*–macrophyte interactions, we first examined daphnid location in our tanks over time using repeated measures analysis of variance (rmANOVA). The percentage of daphnids that occurred in the middle of the tank (where, if present, macrophytes occurred) constituted our repeated measure. We used different statistical analyses to answer a logical sequence of questions. First, we used a one-way rmANOVA to test whether the presence of fish cue changed the percentage of daphnids that occurred in the middle of the tank in the absence of macrophytes (Table 1, part A). We needed to confirm that daphnids did not move into the middle of the tank whenever presented with fish cue but that they did so only in the presence of macrophytes. Second, we tested how daphnids responded to macrophytes in the absence of fish, using a two-way rmANOVA to test how macrophyte type (plastic, real) and density (low, medium, or high %PVI) influenced the temporal pattern of daphnid distribution in the absence of fish (Table 1, part B). To test the multiple effects of fish cues, macrophyte type, and density, we used a three-way rmANOVA (Table 2) to determine whether different macrophyte types (plastic, real) and densities (low, medium, or high %PVI) significantly altered daphnid distribution when different fish cues were present (roach, perch) or absent (no cue).

Table 2. Three-way repeated measures ANOVA (between subject effects) with three treatments: (1) fish cue (absent, roach, perch); (2) macrophyte density (%PVI: low, medium, or high); and (3) macrophyte type (real or plastic). The percentage of daphnids that occurred in macrophytes is the repeated measure over time (15, 30, 45, or 60 min).

Effect	Df	MS	F	P
Fish cue	2	0.444	46.870	0.001
Macrophyte density	2	0.002	0.186	0.831
Macrophyte type	1	0.051	5.417	0.025
Fish cue × macrophyte density	4	0.001	0.088	0.986
Fish cue × macrophyte type	2	0.093	9.809	0.001
Macrophyte density × macrophyte type	2	0.001	0.076	0.927
Fish cue × macrophyte density × macrophyte type	4	0.029	3.078	0.026
Error	42	0.009	—	—

PVI, plant volume infested.

The results of the analyses described above (see Results; Tables 1, 2) led us to test the importance of the presence of fish cues and macrophyte type (independent of macrophyte density) on daphnid behavior using a two-way ANOVA (Table 3). We used difference data in this two-way analysis to represent change in distribution of daphnids as follows: we averaged our 15- and 30-min observations to determine a “before fish” value, then averaged our 45- and 60-min observations for an “after fish” value; subtracting the “before” from the “after” values eliminated the effect of time (Table 3). Following the two-way ANOVA, we used Tukey’s multiple comparison tests (MCTs) to compare the difference statistic among macrophyte types and different fish cues. All analyses were conducted with SAS software (SAS 1994; Cody and Smith 1997).

Influence of macrophyte structure on *Daphnia* mortality from roach and perch—At the conclusion of the behavior observations (described above), we released fish from cages and removed the inner mesh cylinder that separated the open water and macrophytes, allowing fish access to the entire tank. We allowed the fish to forage for 2 h, during which we observed the location of single fish (open water or macrophytes) in each tank at 45, 60, and 90 min during their foraging period. After 2 h we drained each tank to recover remaining *Daphnia* and compared predation on *Daphnia* be-

Table 3. Two-way ANOVA (between subject effects) with two treatments: (1) fish cue (absent, roach, perch) and (2) macrophyte type (real or plastic). Macrophyte densities (%PVI) are pooled. Data are differences (after—before) between the percentage of daphnids that occurred in the macrophytes after fish cue and the percentage of daphnids in the macrophyte habitat before a fish cue was added.

Effect	Df	MS	F	P
Fish cue	2	0.7668	91.07	0.0011
Macrophyte type	2	0.0693	8.23	0.0017
Fish cue × macrophyte type	4	0.9519	11.31	0.0011
Error	61	0.0084	—	—

PVI, plant volume infested.

tween roach and perch and among different macrophyte densities. For a second predation experiment, we set up additional tanks (in a manner similar to that used for previous experiments) to test how well high densities of real (70% PVI) and plastic (94% PVI) macrophytes decreased *Daphnia* mortality against predation by roach and perch together (one fish each). Other methods were identical to those used in the earlier experiments.

For both fish species and both types of macrophytes, we used one-way ANOVAs and Tukey's MCT to test if *Daphnia* mortality differed significantly among levels of increasing PVI. Using another one-way ANOVA, we compared daphnid mortality against different predators (none, roach only, perch only, or both roach and perch) at high macrophyte densities only. Observational data regarding habitat location of roach and perch violated the ANOVA assumption of normality, even after transformations. Therefore, we used a two-way, nonparametric Friedman's test (Zar 1993) to examine habitat use of roach and perch. We tested for significant differences in the habitat choice of our fishes between real and plastic macrophytes using a sign test (Zar 1993).

Results

Preliminary analyses: Behavioral response of *Daphnia* to fish and macrophytes—In the absence of macrophytes, the percentage of daphnids occurring in the middle of the tank did not differ in the absence of fish relative to the presence of caged roach or perch (Table 1, part A; $P = 0.661$; Fig. 1A "none" data versus Fig. 1B,C "none" data). Daphnids preferred the open water. In the absence of fish, the addition of either plastic or real macrophytes also did not change the distribution, as daphnids still preferred the open water. Furthermore, the temporal pattern of daphnid location did not differ among the macrophyte densities (low, medium, and high PVI) in the absence of fish (Table 1, part B; $P = 0.898$). Thus, the three PVI treatments are pooled on Fig. 1 for simplicity. However, macrophyte type ($P = 0.005$) significantly influenced the percentage of daphnids occurring in the middle of the tank over time (Table 1, part B). After 1 h of observation, fewer daphnids occurred in real macrophytes than in plastic ones in the absence of fish (Fig. 1A; Tukey's MCT, $P < 0.05$).

We found that both fish cue ($P = 0.001$) and macrophyte type ($P = 0.025$) strongly influenced the percentage of daphnids that occurred in the middle of the tank, whereas macrophyte density had no effect ($P = 0.831$) (Table 2). A significant interaction occurred between fish cue and macrophyte type together ($P = 0.001$) and also when these factors were combined with macrophyte density ($P = 0.026$). However, regardless of macrophyte density, daphnids did not readily occur in macrophytes in the absence of fish, but daphnids moved into macrophyte habitat when cues from either roach or perch were added (Fig. 1). Fewer daphnids occurred among *Elodea* than plastic macrophytes. Significant interactions involving macrophyte density only occurred when both macrophyte type and fish cue were included in the analysis. No relationship existed between only macrophyte density and fish cue ($P = 0.986$) or between

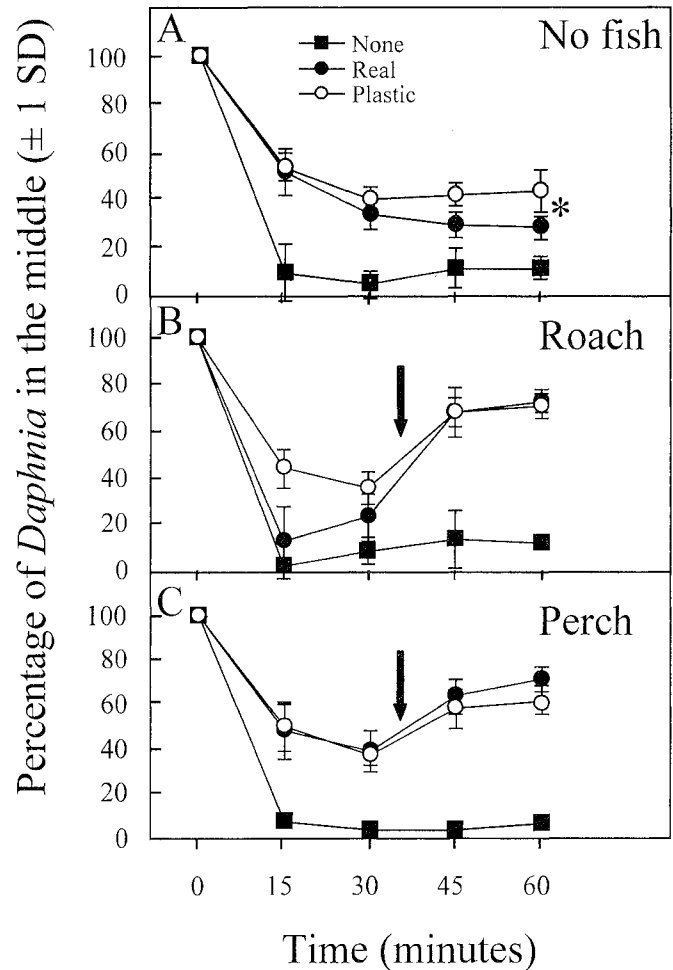


Fig. 1. Influence of macrophytes and caged fish on daphnid behavior. When macrophytes were present, they occurred in the middle of the tank. Data represent the percentage of *Daphnia* choosing to reside in the middle of the tank in the absence of macrophytes, presence of real macrophytes, or plastic macrophytes over time under three treatments of fish cue: (A) no fish, (B) caged roach (*Rutilus rutilus*), and (C) caged European perch (*Perca fluviatilis*). An arrow indicates the time at which a single caged fish was added. * = $P < 0.05$.

macrophyte density and macrophyte type alone ($P = 0.927$). Thus, to further test the roles that macrophyte type and fish cue played in influencing the percentage of daphnids that occurred in the macrophytes, we pooled macrophyte densities (%PVI) into macrophyte type (plastic or real) for our final analysis.

Behavioral response of *Daphnia* to fish and macrophytes—Both the species of fish and the type of macrophyte significantly altered the distribution of daphnids in our lab experiments (Table 3; fish, $P = 0.0011$; macrophyte, $P = 0.0017$). Daphnids remained around the outside of the tank in the absence of macrophytes, regardless of fish (Fig. 1A–C). Only 27% of daphnids occupied habitat with *E. canadensis* in the absence of fish, and significantly more daphnids occupied plastic macrophytes than *E. canadensis* (Fig. 1A). In the presence of caged fish, daphnids shifted from open

water habitat to macrophyte habitat (Fig. 1B,C). Significantly more daphnids (58–72%) occurred in the macrophytes in the presence of roach and perch (Fig. 1B,C; Tukey's MCT, $P < 0.05$) than in the absence of fish (Fig. 1A). Additionally, daphnid location shifted more in the presence of roach than it did in the presence of perch (Tukey's MCT, $P < 0.05$).

Influence of macrophyte structure on Daphnia mortality from roach and perch—Roach predation on *Daphnia* was significantly lower at the higher density of both plastic (Fig. 2A; one-way ANOVA, $F_{3,8} = 11.9$, $P < 0.003$) and real macrophytes (Fig. 2B; one-way ANOVA, $F_{3,8} = 10.2$, $P < 0.004$). We found similar trends in mortality reduction for both real and plastic macrophytes. Mortality at high PVI was about 40% compared to that at zero PVI (Fig. 2A,B). In contrast, perch predation on *Daphnia* was not related to macrophyte density for either plastic (Fig. 2C; one-way ANOVA, $F_{3,12} = 0.55$, $P = 0.66$) or real macrophytes (Fig. 2D; one-way ANOVA, $F_{3,12} = 0.42$, $P = 0.74$). Perch predation caused at least 50% daphnid mortality for every density of plastic and real macrophytes (Fig. 2C,D) but was never as high as roach predation in the absence of structure.

In our second predation experiment, using only high macrophyte densities in the presence of different predators, daphnid mortality increased with the addition of a predator for both plastic (Fig. 3A; one-way ANOVA, $F_{3,9} = 13.1$, $P = 0.001$) and real macrophytes (Fig. 3B; one-way ANOVA, $F_{3,9} = 19.3$, $P < 0.001$). In the absence of predators, we found a very low percentage of mortality (mean \pm 1 SD, $1.7 \pm 3.3\%$ and $2.8 \pm 3.4\%$ for plastic and real macrophytes, respectively). At these high macrophyte densities, the addition of roach to plastic or real macrophytes did not significantly increase daphnid mortality compared with the control (Fig. 3A,B; Tukey's MCT, a versus b, $P = 0.26$ versus 0.37, respectively). However, daphnids suffered significantly higher mortality in the presence of perch than in the absence of fish for both plastic and real macrophytes (Tukey's MCT, a versus c, $P = 0.02$, 0.004, respectively). Daphnid mortality did not differ between the treatment with only one perch versus one perch and one roach (Fig. 3A,B; Tukey's MCT, bc versus c, $P = 0.10$ for plastic and real macrophytes).

In our laboratory experiments, we averaged location observations over time for both single roach and single perch. Perch occupied macrophytes more frequently than did roach (Fig. 4; Friedman two-way ANOVA, Friedman (χ^2)_{0.05,3} = 21.3, $P < 0.001$). Examining the influence of macrophyte type on habitat choice showed that roach equally frequented plastic and real macrophytes (sign test, $P = 0.75$). In contrast, perch spent more time in real macrophytes relative to plastic ones (sign test, $P = 0.04$).

Discussion

A paradox exists between recent studies, which indicate that macrophytes provide daytime refuge for *Daphnia* against fish predation (Moss et al. 1998), and historical documentation of daphnids, which shows that they avoid vegetation (Hasler and Jones 1949; Pennak 1973). Yet despite the importance of *Daphnia* in aquatic systems (de Bernardi and Peters 1987), only a few studies have attempted to

solve this paradox (Lauridsen and Lodge 1996). In experiments without fish, we showed that fewer daphnids occupied real versus plastic macrophytes. These results are consistent with previous suggestions that repellent chemicals from aquatic vegetation may partially explain the reasons for which macrophytes deter daphnids (Lauridsen and Lodge 1996). We confirmed the overriding importance of fish cues versus macrophyte cues, as daphnids greatly increased their use of macrophyte habitat in the presence of planktivorous fishes, regardless of fish species.

We also demonstrated that submerged macrophytes provided critical daytime refuge for *Daphnia* against predation by some planktivorous fishes. Considered together, our results offer a resolution to the paradox, allowing us to put previous work, most of which was focused only on *Daphnia* response to macrophytes or only on *Daphnia* response to fishes, in a more holistic context. Overall, *Daphnia* behavior appears to reflect an adaptive balance between avoiding littoral habitats (where phytoplankton resources may be scarce or where littoral predators may be abundant) and avoiding pelagic planktivorous fishes during the day, when vulnerability is maximized. Below we discuss past work in this context.

Previous studies (Winfield 1986; Diehl 1988; Swisher et al. 1998) tested the effectiveness of littoral structure mimics (gauze leaves, wooden dowels, nylon cords, and plastic stems) in reducing foraging rates of planktivorous fishes. However, few previous studies have directly tested the ability of real macrophytes or imitation macrophytes to decrease *Daphnia* mortality from fish predation under conditions of daytime light. It is critical to examine the ability of macrophytes to provide refuge against predation under conditions of daytime light, as daytime is the time when daphnids in the field would be most likely to seek refuge in macrophytes (Lauridsen and Buenk 1996). In our experiments, fish consumed almost all daphnids in the absence of any refuge. However, in the presence of refuge, *Daphnia* mortality declined with increasing structural complexity when daphnids were faced with predation by roach. High densities of *E. canadensis* and plastic *Myriophyllum* reduced daphnid mortality from predation by roach by at least 40% during our 2-h experiment. In contrast to experiments with roach, the presence of real or plastic macrophytes, even at high densities, did not lower the success of perch foraging. These results support previous studies showing that European perch effectively forage on *Daphnia*, even at artificial stem densities of 600 m⁻² (Winfield 1986), which is probably roughly equivalent to our 70% PVI, considering the height of the plants. In our experiments, perch spent significantly more time foraging in macrophyte habitat than did roach, and perch consumed up to 80% of the daphnids present. These results indicate that the presence of high densities of perch in lakes may nullify any daytime refuge for *Daphnia*, even in dense macrophytes (Jeppesen et al. 1998). Yet in our experiments, the presence of macrophytes protected daphnids from predation by roach, which foraged less efficiently among structure.

Much debate, however, still exists with regard to the mechanisms of the refuge effect imparted to *Daphnia* by littoral zone structures. Some studies indicate that macro-

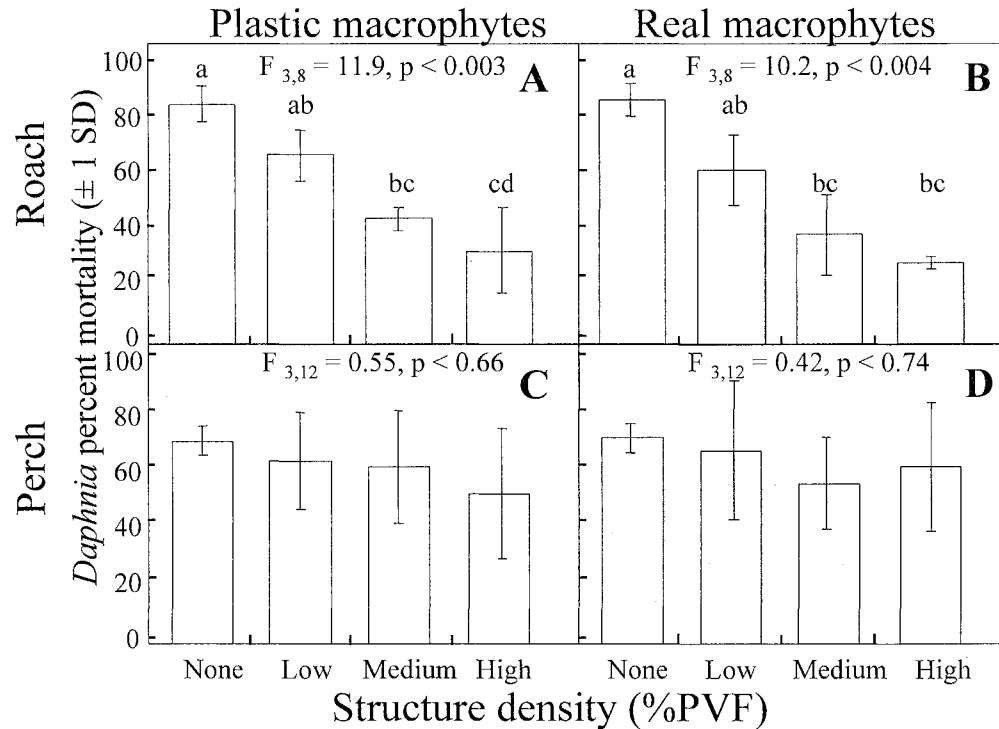


Fig. 2. Left-hand panels depict relationships between plastic macrophyte density and *Daphnia* mortality from (A) roach predation or (C) perch predation. Right-hand panels show real macrophyte density and *Daphnia* mortality from (B) roach or (D) perch. F stats and P values come from one-way ANOVAs. Small letters above bars indicate statistical differences between macrophyte densities (Tukey's MCT).

phyte beds develop conditions, such as low dissolved oxygen (DO) or high pH (Beklioglu and Moss 1995), that deter fish from foraging. Hartleb and Haney (1998) showed that cold temperatures (10°C) and low light conditions, such as those that exist under some macrophyte canopies, decreased foraging activity of small bluegill. Perch already forage less efficiently under conditions of total darkness (Diehl 1988), so we purposely provided adequate light for foraging to test only the ability of macrophytes to provide refuge. Furthermore, in our experiments that were conducted at moderate temperatures (20°C) and during daylight hours, differences in DO or pH did not exist, and littoral zone structures still decreased capture efficiencies of roach during the day. Thus, although abiotic factors may play a role in the field, changes in DO, pH, light, or temperature cannot explain the mechanism behind the refuge effect found in our laboratory studies.

Besides abiotic factors, behavior-mediated interactions between planktivorous and piscivorous fishes may partly account for the mechanism behind macrophyte refuge seen in field studies. A high abundance of planktivores in the vegetation may reflect that pelagic piscivores force planktivores to hide within macrophytes (Persson 1993), thereby diminishing the refuge for daphnids. On the contrary, littoral-associated piscivores that frequent vegetated areas, such as pike, may instead force planktivorous fishes to stay in the open water, thus enhancing littoral refuge for zooplankton (Jacobsen and Perrow 1998). Stansfield et al. (1997) also

point out that macrophytes inhabited by fish can still provide a refuge for large-bodied cladocerans if fish do not feed while hiding. Invertebrate abundance also often increases with increasing macrophyte density (Kornijów and Kairesalo 1994), and refuge may be maintained if fishes choose alternative food sources (Diehl 1992). Our results indicate that without alternative food sources or threatening piscivores, small perch efficiently capture *Daphnia*, even among high macrophyte densities. However, our lab experiments may have overestimated the impact that perch predation could have on daphnids in the field, where other food resources are available.

If littoral zone structures can decrease daytime predation rates on daphnids and daphnids avoid macrophytes in the absence of fish (Hasler and Jones 1949), it logically follows that daphnids must sense a signal from predators that prompts their movement into refuge during the day. Lauridsen and Lodge (1996) showed that daphnids behaved similarly when exposed to a caged fish or to water that previously held a fish. We used only caged fish in our experiments. Although visual stimuli may partly influence daphnids, previous experiments indicate that daphnids respond largely to fish chemical cues (Macháček 1991). With regard to DHM in shallow lakes, it is crucial that investigations explore the interaction of fish chemical cues with macrophytes cues to understand when daphnids are likely to seek refuge in macrophytes.

Our laboratory experiments confirmed that daphnids

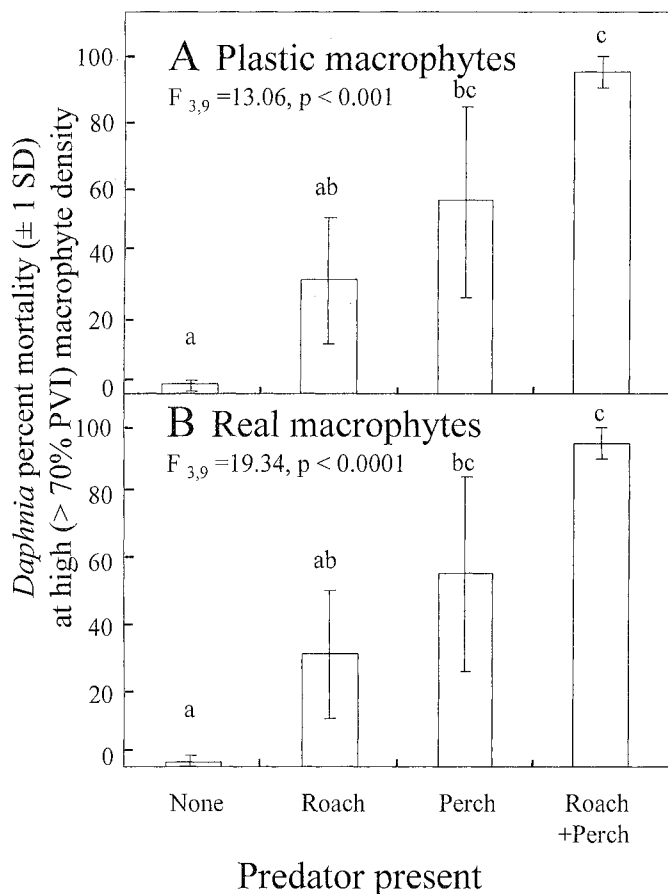


Fig. 3. Comparison of *Daphnia* percent mortality within high densities of (A) plastic (94% PVI) or (B) real (70% PVI) macrophytes under different predation pressures: no predation ($N = 3$), single 1+ roach ($N = 3$), single 0+ perch ($N = 4$), or both 1+ roach and 0+ perch ($N = 3$). F stats and P values are from one-way ANOVAs. Small letters above bars indicate significant differences in daphnid mortality between experiments.

greatly increased their use of macrophyte habitat only in the presence of fish, regardless of fish species. In rectangular tanks, daphnids often congregate in the corners. We minimized this common tank effect by using circular tanks. However, for reasons unknown, in the absence of macrophytes and fish, daphnids preferred the outer ring of the tank. Despite its frequency, few explanations are offered for this behavior artifact from daphnids in laboratory experiments (de Bernardi and Peters 1987), although response to lighting conditions is the most plausible explanation (Ringelberg 1987). Interestingly, this behavioral artifact strengthens our results. Daphnids moved into the middle of the tank, despite their natural tendencies. Our results from treatments without macrophytes verify that daphnids do not simply move into the middle of the tank when a fish cue is added. Macrophytes must be present to elicit that response, and daphnids only seek refuge when macrophytes occur. Daphnids do not swim into the middle simply as an escape mechanism. In the absence of fish, only 27% of daphnids occurred among the *E. canadensis*. However, in the presence of fish, over 50% of daphnids chose to seek protection. If daphnids exhibited ran-

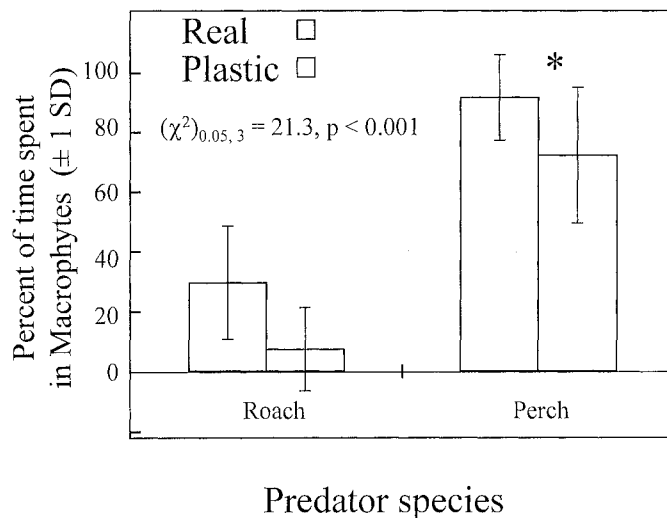


Fig. 4. Percent of time spent in plastic or real macrophyte habitat by single roach or single perch during the predation time of experiments. χ^2 statistic and P value result from Friedman two-way ANOVA. An * indicates a significant difference (from a sign test) in time spent between real and plastic macrophytes.

dom behavior, we would have expected that only 36% of the daphnids would have occurred by chance in the macrophytes (macrophytes occupied 36% of the arena volume). Additionally, by the end of the experiments, significantly fewer daphnids occurred among real *Elodea* than among plastic *Myriophyllum*, in the absence of fish. Therefore, prolonged exposure to macrophyte chemicals elicits less use of real versus plastic macrophytes.

Following the addition of fish cues, daphnids no longer responded differently to real and plastic macrophytes. As Lauridsen and Lodge (1996) first demonstrated, the presence of fish cue overrides any repellent effect of macrophyte chemicals. We add to those results by demonstrating that daphnids do not discriminate between cues from roach and perch, despite the different foraging strategies of these fishes. Because roach often remain in open water habitats in the absence of piscivores (Persson 1993), whereas perch are superior foragers among vegetation (Winfield 1986), we expected *Daphnia* to choose macrophyte habitat more in the presence of roach than in the presence of perch. However, daphnids entered macrophytes equally in the presence of either roach or perch.

Our results provide the first experimental evidence linking daphnid movement into littoral structures with reduced mortality from daytime predation. In shallow lakes, daphnids need to seek refuge from planktivorous fishes during the day, when light often penetrates to the sediment surface. We examined *Daphnia* behavior over short time and spatial scales, but both important behaviors (avoidance of real macrophytes and entering macrophytes in response to fish) happened rather quickly. Although investigated on small spatial and temporal scales, we believe that our results provide important mechanistic understanding related to the littoral refuge effect and help resolve the existing paradox between daphnid use of and avoidance of macrophytes.

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