Macrophyte and fish chemicals suppress *Daphnia* growth and alter life-history traits

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Daphnids undergoing diel horizontal migration (DHM) to seek daytime refuge in the littoral zones of shallow lakes are likely to confront chemical cues from littoral-associated predators and macrophytes. In field experiments, we investigated how the natural suite of chemicals occurring in a wholly vegetated lake as well as within plant-free mesocosms with artificial macrophytes and epiphytes (either fishless or containing small fish) influenced individual daphnid growth. In laboratory experiments, we further examined how water containing chemicals from either a submerged macrophyte (waterweed, *Elodea canadensis*), a planktivorous fish (roach, *Rutilus rutilus*) or both impacted daphnid growth and life-history traits. In the field, we found the greatest suppression of daphnid growth in vials containing water from the wholly vegetated lake relative to growth of daphnids housed in vials containing spring water. Water from the mesocosm with fish also suppressed daphnid growth. Daphnid growth in water from the fishless mesocosm, which contained plastic plants colonized by epiphytes, did not differ from that of daphnids grown in spring water. In the lab experiment, daphnids exposed to *Elodea* chemicals took longer to mature and possessed fewer eggs than daphnids in media without *Elodea* chemicals. Daphnids exposed to chemicals from both *Elodea* and roach reproduced the earliest and at a smaller size. Daphnids exposed to only roach chemical cues did not significantly differ from daphnids in control media for age or size at first reproduction although they did possess fewer eggs. *Daphnia* responses to chemicals from either roach or *Elodea* alone did not predict how *Daphnia* responded to the combined influence of multiple chemical cues. Our results suggest that prolonged exposure to macrophyte chemicals incurs costs for *Daphnia*.

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Chemical cues from planktivorous fish and invertebrate predators induce large herbivorous zooplankton, such as *Daphnia*, to undergo diel vertical migration (DVM) into dark, cold waters of stratified lakes for refuge (Vanni 1987, Lampert 1993, Von Elert and Loose 1996). The hypolimnetic refuge often allows *Daphnia* to co-exist with fish or other predators, and results in a lower phytoplankton biomass than would exist in the absence of *Daphnia* (Edmondson and Litt 1982). Due to their large body size and high vulnerability to predation (Brooks and Dodson 1965), daphnid survival may

depend on such behavioral responses to chemical cues from predators. Increasing evidence suggests that this may apply to littoral as well as pelagic habitats (Lauridsen and Buenk 1996).

Ecologists often perceive daphnids as being purely pelagial (Hrbaček et al. 1961, de Bernardi and Peters 1987, Carpenter and Kitchell 1993), but increasing evidence suggests that littoral habitats can also be important to *Daphnia* survival (Lauridsen et al. 1996, Moss et al. 1998). Despite early studies that showed *Daphnia* avoid macrophytes (Hasler and Jones 1949, Pennak

1973), *Daphnia* sometimes aggregate in littoral zones of shallow lakes (Lauridsen and Buenk 1996, Lauridsen et al. 1998), especially in dense plant beds (Jeppesen et al. 1997). In shallow systems, where DVM is less advantageous, *Daphnia* may migrate into littoral vegetation to seek refuge in macrophytes during the day, an alternative predator-avoidance strategy termed diel horizontal migration (DHM) (Timms and Moss 1984, Kvam and Kleiven 1995, Lauridsen and Buenk 1996, Stansfield et al. 1997). DHM may allow *Daphnia* populations to persist, and potentially reduce algal biomass in some shallow lakes (Lauridsen et al. 1996, Perrow et al. 1997, Jeppesen et al. 1998) but not all (Lauridsen et al. 1999).

Moving between pelagic and littoral zones, daphnids may confront multiple chemical cues. The ability of daphnids to differentiate between these multiple cues may play a decision-making role in habitat choice. In this context, 'chemical cue' can be defined as an infochemical. An infochemical is a chemical, which in its natural context, conveys information between two organisms. This information evokes a response that is adaptive for one or both organisms (Dicke and Sabelis 1988). Except for isolation of allelopathic (those that inhibit growth) substances exuded by cyanobacteria (Gross et al. 1991), actual identification of specific chemical compounds is rare for interactions among freshwater organisms. Although only basic properties are known for fish chemical cues (Von Elert and Loose 1996), the presence of these cues evokes a variety of prey responses.

Predator-derived chemicals (for reviews, see Larsson and Dodson 1993, Kats and Dill 1998) prompt *Daphnia* to migrate away from predators, both vertically (De-Meester 1993, Weider and Pijanowska 1993, Reede and Ringelberg 1995) and horizontally (Watt and Young 1994, Kleiven et al. 1996, Lauridsen and Lodge 1996). Chemicals from predators also influence a wide range of life-history traits including size and age at first reproduction and clutch size (Machaček 1991, Stibor 1992, Sakwiñska 1998). Moreover, changes in morphology can occur including helmet extension, tail spine elongation and neck-teeth production (Dodson 1988a, b).

If *Daphnia* are prompted by predator chemical cues to undergo DHM, it is important to understand how chemical cues from the littoral zone environment affect *Daphnia* behavior, growth and life-history traits. In one study (Lauridsen and Lodge 1996), daphnids exhibited an aversion to macrophytes which was overcome by the presence of fish or fish odor. Macrophytes also release chemical substances that deter phytoplankton (Jasser 1995) but it is unknown how these chemicals directly, or indirectly, impact higher trophic levels. Furthermore, epiphytes often colonize macrophytes and may exude their own chemicals (Phillips et al. 1978). Few studies have tested the combined impact of chemicals from epiphytes, macrophytes and predators on *Daphnia* behavior, growth or life-history traits.

In two separate field experiments, we tested the prediction that chemicals associated with littoral zones would suppress daphnid growth. To further examine the impact of chemical cues on life-history traits, we exposed daphnids to chemical cues from a submerged macrophyte (*Elodea canadensis*) and a common predatory fish (*Rutilus rutilus*), both alone and in combination, in a laboratory experiment. Consistent with Reede (1995), we expected that daphnids would alter their life-history strategies and reproduce earlier at a smaller size when exposed to multiple chemical stresses.

Methods

Field experiment

We collected *Daphnia magna* from Lake Ring in Denmark and isolated individuals between 750 and 900 μm (carapace length from the top of the head to the base of the tail spine) by filtering water through different sized mesh. We performed two experiments to expose *Daphnia* to different types of chemical cues and examine their growth response. The first experiment took place in the littoral zone of wholly vegetated Lake Stigsholm (Table 1), which contained chemical cues from macrophytes, epiphytes and fish. The second experiment took place in two mesocosms located in Lake Torup (Table 1). Within these mesocosms, we could better isolate chemical cues from fish, epiphytes, or both.

Before each experiment, we fixed a random sample of daphnids (25 for the 1st experiment and 15 for the 2nd experiment due to lower availability of daphnids within the correct size range) in acid Lugol's solution and measured their length to determine a mean initial size. At the conclusion of the experiment, we collected and narcotized individuals with soda water to preserve carapace shape, placed them in a 1.5-mL siliconized microtubule with acid Lugol's solution and later measured carapace length. We calculated mean daily growth ((final size-initial size)/time) for each treatment.

For each experiment, we incubated individual *D. magna* females in spring water or filtered (0.45 μm GFF) lake water collected from the site of the experiment. To allow measurement of individual growth, we housed single daphnids in closed, impermeable polyethylene vials (55 mL) that were weighted and suspended from rods at a depth of 0.5 m. We conducted these experiments in the field to expose daphnids to ambient lake conditions. We deliberately chose closed, impermeable vials instead of flow-through vials to isolate the effect of chemical cues on daphnids and control their food resource.

Table 1. Characteristics of two field sites: A = area (hectares for whole lake, m² for whole mesocosm and inner ring () of macrophytes); z_m = maximum depth (m); z_{ave} = average depth (m); TP = mean summer total phosphorus (μg L⁻¹) range for whole lakes or average TP for mesocosms on 28 July 1997+1 SE; T = average daytime temperature (°C)+1 SE and range at surface and bottom during experiment; DO = average daytime dissolved oxygen (mg L⁻¹)+1 SE and (range) at surface and bottom during experiment; pH = daytime average; macrophytes = species and %PVI (plant volume infested); cyanobacteria present = dominant species present; total chl a = average chlorophyll a concentration (μg L⁻¹+1 SE) during experiment; dominant fish at site (per m² if available): pike (Esox lucius), perch (Perca fluviatilis) and roach (Rutilus rutilus).

Site	A	z_m	z_{ave}	TP	$T_{surface}$ T_{bottom}	$DO_{surface}$ DO_{bottom}	pH	Macrophytes	Cyanobacteria Total Chl a	Dominant fish
Lake Stigsholm	21	1.2	0.8	100–150	15.3+0.4 (13.9–16.5) 14.7+0.3 (13.9–15.7)	14.6+1.3 (11.3–18.3) 14.2+1.5 (11.4–19.6)	9.1	Elodea canadensis >90% Potamogeton pusillus <5% P. crispus <5% P. pectinatus <5%	Microcystis sp. 18.2+2.5	Pike Perch Roach
Lake Torup	18	7	4.4	50–150						
Mesocosm without fish	78 (20)	1.7	1.5	105+17	21.9+0.0 (21.4–22.5) 21.3+0.0 (20.3–21.6)	10.2+0.1 (8.9–10.8) 9.6+0.1 (9.1–10.0)	8.0	Plastic macrophytes Inner ring: 20, 40, 80	Planktothrix agardii 6.3+0.7	0
Mesocosm with fish	78 (20)	1.7	1.5	155+33	21.8+0.1 (21.2–22.5) 21.8+0.1 (20.3–21.6)	10.3+0.2 (8.0–10.9) 9.7+0.1 (8.8–10.2)	7.9	Plastic macrophytes Inner ring: 20, 40, 80	Planktothrix agardii 5.8+0.5	Roach (4)

For both experiments, we placed daphnids into two treatments. The control treatment consisted of an individual daphnid housed in a closed vial containing only spring water and 10 mL of a culture of *Scenedesmus* (mean ± 1 SE; 5300 cells mL⁻¹ ± 117 SE; 173 μg chl *a* L⁻¹). The chemical cue treatment consisted of an individual daphnid in filtered lake water (0.45 μm GFF) with a quantity of *Scenedesmus* equal to that in the control. We renewed either spring water (control) or filtered lake water (chemical cue treatment) and algae daily for each vial to avoid colony formation or spine elongation of *Scenedesmus* (Hessen and Van Donk 1993, Lampert et al. 1994). Clumps of algae did not accumulate in the vials. For the second experiment, we measured chlorophyll *a* (ethanol extraction) left in each vial at the conclusion of the experiment with a spectrophotometer (Jespersen and Christoffersen 1987). For the chemical cue treatment, we collected water to filter each day from the location where each set of vials occurred. We also measured daytime temperature, pH and dissolved oxygen at the surface and bottom of the water column daily using calibrated probes (YSI Model 58).

In Lake Stigsholm, we placed twenty vials ($N = 20$) of each treatment within dense beds of *Elodea canadensis* (naturally colonized by epiphytes) and incubated them for four days (25–28 June 1997). In Lake Torup, we conducted the experiment in two cylindrical mesocosms constructed of impermeable plastic, which extended approximately 5 cm into the lake sediment (Fig. 1). Two months before the experiment, we removed all natural macrophytes (*Polygonum amphibium* L.) from both mesocosms. Thereafter, an inner circle (5-m diameter, not surrounded by any barrier) in each mesocosm was supplied with plastic macrophytes (imitation ivy) at three densities (20, 40, and 80% plant volume infested, Canfield et al. 1984); each density occupied a
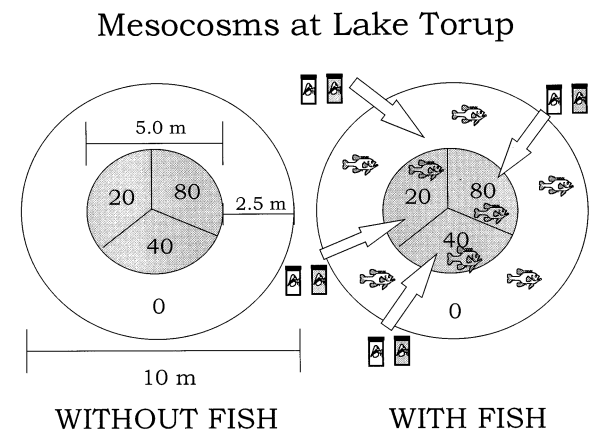
Mesocosms at Lake Torup

Fig. 1. Schematic of Torup mesocosms. Six replicates of both control (open) and treatment (shaded) vials occurred in each section of each mesocosm, partially shown only on the right side of the figure. Shaded sections represent areas of plastic macrophytes (colonized by epiphytes). Numbers refer to percent plant volume infested (%PVI) of plastic macrophytes.

wedge-shaped area of 6.5 m² (Fig. 1). An outer ring of open water (0% PVI) surrounded the inner circle of plastic macrophytes. Fish could move freely between vegetated and open water sections. We stocked one mesocosm with 0 + roach (*Rutilus rutilus*) and kept the other mesocosm fishless. Epiphytes occurred on the plastic macrophytes. We placed six vials of both the control and chemical cue treatment in all four sections (0, 20, 40, 80% PVI) of both mesocosms (fishless and with fish). Thus, the Lake Torup experiment involved placing our control and chemical cue treatments ($N = 6$) into eight combinations of plastic macrophytes and fish. The experiment also ran for four days (11–15 July 1997).

We first tested our data from both experiments for normality using Kolmogorov and Smirnov (KS) tests. Final sample numbers in our experiments were less than initial because we lost some vials due to handling error (five spring water, three filtered water in Lake Stigsholm; three spring water, four filtered water in Lake Torup fishless mesocosm; two filtered water in mesocosm with fish). We then used a *t*-test to compare growth between the control and chemical cue treatments incubated within the wholly vegetated lake. For the Lake Torup mesocosms, we used a two-way ANOVA to test for differences in growth of daphnids in water collected from the different plastic macrophyte densities in the mesocosms with or without fish. If no significant differences in daphnid growth existed between the different macrophyte densities, we pooled the data. To test for the effect of epiphytes, we compared daphnids in vials containing filtered water from vegetated areas in the fishless mesocosm with daphnids grown in spring water using a *t*-test. To test for a fish effect, we compared the growth of all daphnids from the mesocosm without fish against all daphnids from the mesocosm with fish using a *t*-test. To compare the influence of different chemical combinations, we used a one-way ANOVA followed by a Tukey's Multiple Comparison Test (MCT).

Laboratory experiment

To test how the natural suite of chemicals from a specific macrophyte and fish influenced *Daphnia* growth and life history strategies, we prepared four 'chemical treatments': 1) COMBO medium (Kilham pers. comm.) as a control or COMBO medium (10 L) that housed either 2) 75 g (wet weight) of *Elodea canadensis*, 3) a single age 1 + roach (*Rutilus rutilus*) (10 ± 0.5 SE g wet weight) or 4) both *Elodea* and roach. Four to six hours before adding them to the media, we fed roach flake food (which did not contain dried *Daphnia*) instead of *Daphnia* to avoid potential chemical cues from crushed-up conspecifics (Pijanowska 1997). We did not feed fish while they were held in the media, and we replaced fish and *Elodea* every 2–3 d. Every 2 d, we removed 2.5 L of media to filter for the chemical treatments only after

the macrophyte or fish had been in the media for 24–36 h. We then added 2.5 L of plain COMBO media. For this experiment, we isolated newly released, *Daphnia pulex* neonates from the 3rd clutch of parthenogenetically reproducing females, which were originally collected from Lake Engelsholm, Central Jutland, Denmark. Lake Engelsholm contains both *E. canadensis* and roach.

We kept single *D. pulex* in 250-mL glass beakers containing 200-mL of filtered (0.45 µm GFC) COMBO media from the four prepared stocks. The experiment consisted of 10 replicates (individual daphnid in 250-mL beaker) per treatment kept in a climate-controlled room with a temperature of 20 (± 1)°C and a photoperiod of 16:8 L:D. Every day, we fed each daphnid *Scenedesmus acutus* (1 mg C L⁻¹) that had been cultured in plain COMBO media. To assess growth, we measured carapace length of each daphnid every 2–3 d using an image analyzer and stereomicroscope (25 ×).

To measure individuals, we carefully removed individual daphnids from their beaker with a plastic pipette. We positioned an individual daphnid in a large drop of water on 20-µm mesh that we then slid into the viewing area of the microscope. The image was then projected onto a computer screen that facilitated measurement of the carapace. To address life-history traits, we recorded size and age at first reproduction and number of eggs produced. After each measurement, we checked to make sure that air had not been trapped under the carapace of the daphnids. We then replaced the daphnids in newly filtered media containing the chemical cues.

The experiment ceased after all individuals produced eggs, at which time we made final measurements for length. We lost four replicates during the experiment due to daphnid mortality (one from the control, one from media with *Elodea*, two from media with *Elodea* and roach). We used a repeated measures ANOVA (rmANOVA) analysis to examine daphnid growth through time. We also used one-way ANOVAs followed by Tukey's multiple comparison tests (MCTs) to test for differences among treatments in final daphnid size, age and size at reproduction and number of eggs.

Results

Field experiments

Initial size of daphnids did not differ between experiments (*t*-test, $p = 0.618$). Data from both experiments were normally distributed (KS test, Lake Stigsholm, Lilliefors $p = 0.644$, Lake Torup, $p = 0.698$). Daphnids grown in water filtered from Lake Stigsholm (with naturally occurring macrophytes, epiphytes and fish, Table 1) grew significantly less than those in spring water (Fig. 2A; *t*-test, $p < 0.001$). After 4 d, daphnids in
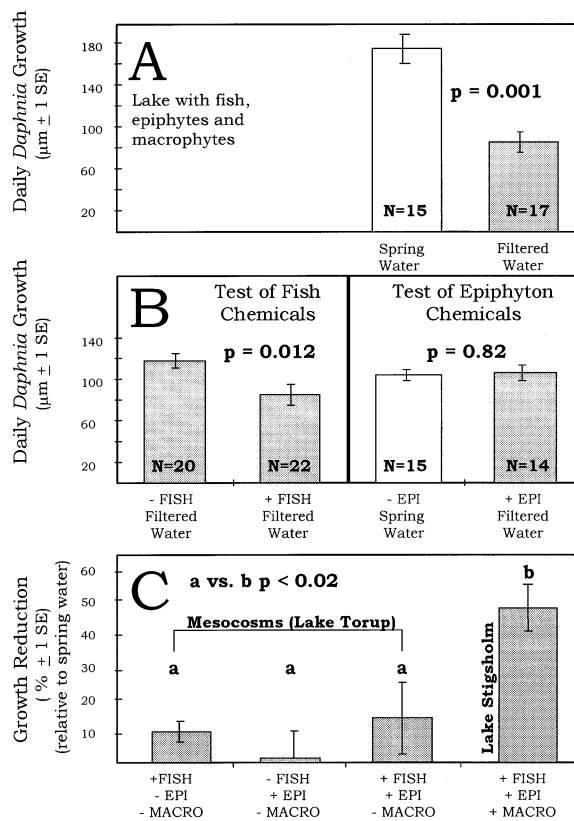
Fig. 2. Impact of chemicals from wholly vegetated lake (Lake Stigsholm) (A) and from nearshore mesocosms (Lake Torup) (B) on *Daphnia* daily growth, with a comparison of the magnitude of growth reduction resulting from exposure to different chemical cues in the two experiments (C). Open bars (A, B) = daphnids grown in spring water. Shaded bars = daphnids housed in filtered water. For A and B, *p*-values are from *t*-tests. For C, small letters above bars indicate results from Tukey's MCT. Also for C, daphnids in filtered and spring water are incubated at the same location: open water section of mesocosms with fish (1st bar), area of the mesocosm vegetated with plastic plants without fish (2nd bar), area of the mesocosm vegetated with plastic plants with fish (3rd bar), wholly vegetated lake (4th bar). EPI = epiphytes present. MACRO = real macrophytes. Presence and absence of cue denoted by + and −, respectively.

spring water were nearly twice the size of daphnids kept in filtered water from the wholly vegetated lake. Neither reproduction nor mortality occurred in any of the vials.

For both mesocosms at Lake Torup, no differences existed in growth of daphnids housed in water collected from the different plant densities (two-way ANOVA, $F_{2,24} = 1.237$, $p = 0.308$). We therefore pooled daphnids from the different macrophyte densities to test for the effect of fish. Daphnids held in filtered water taken from the fishless mesocosm grew significantly faster than daphnids in filtered water from the mesocosm containing fish (Fig. 2B, left side; *t*-test, $p = 0.012$).

We also compared the growth of daphnids in filtered water from the vegetated areas of the fishless mesocosm

with daphnids in spring water to isolate the potential effects of epiphytes (Fig. 2B, right side). Growth of daphnids in filtered water from the vegetated areas did not differ from that of daphnids in spring water (Fig. 2B, right side; *t*-test, $p = 0.82$). In the Lake Torup experiment (Fig. 2B), reproduction did not occur in any treatment and all daphnids survived.

In both experiments, all daphnids received the same food daily. No significant differences in chlorophyll *a* concentrations (after the final 24 h of the experiment) existed between vials containing spring water or filtered water in either mesocosm (*t*-tests, $p = 0.87$ without fish, $p = 0.92$ with fish). To compare results from Lake Stigsholm (Fig. 2A) and the mesocosms from Lake Torup (Fig. 2B), we calculated, for each experiment, the percentage growth reduction in different treatments relative to daphnids held in spring water in that experiment. Daphnids held in filtered water from the mesocosms experienced reductions in growth ranging from approximately 0–20%, while daphnids held in filtered water from the wholly vegetated sustained a mean reduction in growth of nearly 50% (Fig. 2C). Bars depicted in Fig. 2C represent daphnids in filtered water from a particular location relative to control daphnids held in spring water at that same location. Significantly greater suppression of daphnid growth occurred in the wholly vegetated lake experiment than in the mesocosm experiment (one-way ANOVA, $F_{3,66} = 5.059$, $p = 0.003$, Tukey's a versus b, $p < 0.026$).

Lab experiment

Daphnids exposed to different chemical cues began at the same size (Fig. 3; one-way ANOVA, $F_{3,36} = 0.462$, $p = 0.71$). Over time, our chemical cue treatments sig-
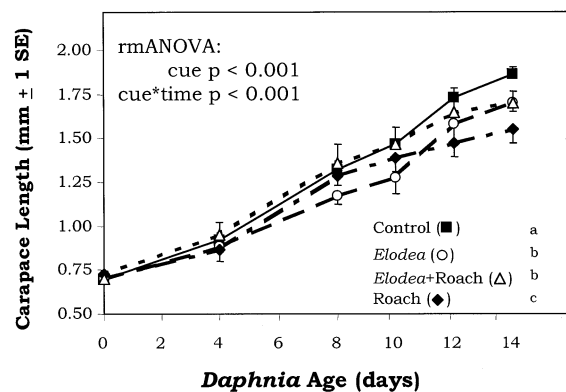

Fig. 3. Repeated measurements of daphnid carapace length in four chemical cue treatments: control with no chemicals (solid line, ■), exposure to *Elodea canadensis* chemicals only (dashed line, ○), exposure to roach chemicals only (dashed and dotted line, ◆) or exposure to chemicals from both *Elodea* and roach (dotted line, △). *p*-values are from rmANOVA from Day 0 to Day 14. Small letters next to treatments indicate results from Tukey's MCT.
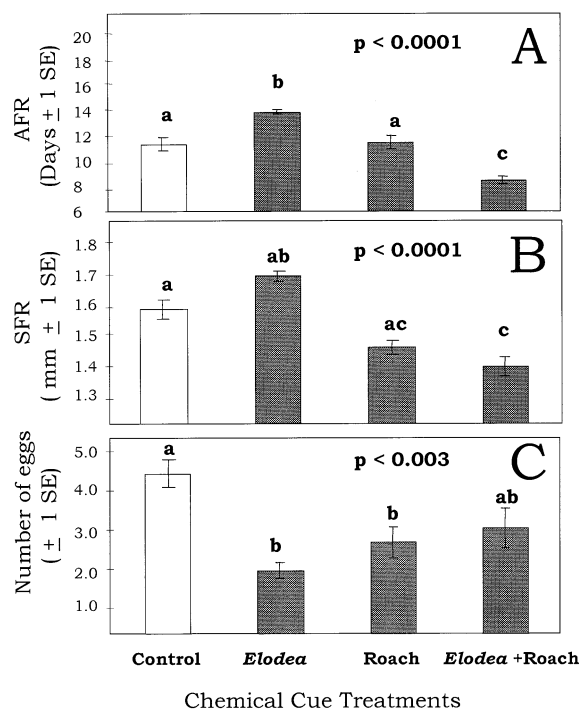
Fig. 4. Influence of chemical cues (none, *Elodea* only, roach only, or both) on daphnid life history parameters: (A) age at first reproduction (AFR), (B) size at first reproduction (SFR), and (C) brood number (number of eggs). Open bar represents the control treatment. Shaded bars represent chemical cue treatments. *p*-values are from one-way ANOVAs. Small letters above bars indicate significant differences from Tukey's MCTs.

nificantly influenced daphnid growth (Fig. 3; rmANOVA, cue $F_{3,32} = 27.00$, $p < 0.001$, cue × time $F_{15,160} = 7.704$, $p < 0.001$). By Day 8, we found smaller daphnids in the treatment with chemical cues from only *Elodea*. However, after Day 10, daphnids exposed to only roach cues replaced daphnids exposed to only *Elodea* cues as the smallest. This trend continued until the end of the experiment (Fig. 3).

All daphnids had produced eggs by Day 14 and final sizes differed significantly between treatments (one-way ANOVA, $F_{3,32} = 31.42$, $p < 0.001$). Final daphnid size in the control significantly exceeded that of all other treatments (Fig. 3; Tukey's MCT, a versus b, c, $p < 0.001$). Final daphnid size did not differ between the *Elodea* only treatment and the *Elodea* and roach treatment (Tukey's MCT, $p = 1.0$). Daphnids in the media with roach chemical cues ended up significantly smaller than both daphnids in the *Elodea* treatment and the *Elodea* plus roach treatment (Fig. 3; Tukey's MCT, b versus c, $p < 0.001$).

Besides differences in growth, significant differences existed between treatments in age at first reproduction (AFR, Fig. 4A) (one-way ANOVA, $F_{3,32} = 21.49$, $p < 0.0001$). Size at first reproduction (SFR, Fig. 4B) (one-

way ANOVA, $F_{3,32} = 16.18$, $p < 0.0001$) and number of eggs (Fig. 4C; one-way ANOVA, $F_{3,32} = 5.785$, $p < 0.003$) also significantly differed between treatments. Daphnids exposed only to *Elodea* chemicals took longer to develop (Fig. 4A) than daphnids isolated from chemicals, but matured at nearly the same size (Fig. 4B). Despite maturing at the same size, daphnids exposed to *Elodea* chemicals possessed significantly fewer eggs (Fig. 4C) than daphnids in the control treatment. Daphnids exposed to *Elodea* chemicals alone matured later and at a larger size than daphnids exposed to roach chemicals alone or in combination with *Elodea* (Fig. 4A, B).

Daphnids exposed to chemicals from roach alone possessed fewer eggs than the control (Fig. 4C) but did not differ in AFR (Fig. 4A) or SFR (Fig. 4B). Daphnids exposed to both *Elodea* and roach reproduced earlier and at a smaller size than those isolated from chemicals (Fig. 4A, B), but did not differ in egg number (Fig. 4C). Egg number did not significantly differ among the three chemical cue treatments.

Discussion

Our results supported the hypothesis that growth was depressed for daphnids exposed to the natural suite of chemicals occurring in littoral zones. In two separate field experiments, we demonstrated that daphnids held in filtered lake water grew less than daphnids held in spring water. Our experiment within the Lake Torup mesocosms illustrated that fish chemical cues impact short-term growth in addition to behavior (DeMeester 1993, Lampert 1993, Lauridsen and Lodge 1996) and morphological traits (Dodson 1988a, b). These results agree with previous studies showing that daphnids are smaller in the presence of fish cues (Machaček 1991, Reede 1995), which is most likely an adaptation to diminish the likelihood of predation (Brooks and Dodson 1965). Growth suppression did not occur for daphnids grown in vials with filtered water from the areas containing epiphyton within the fishless mesocosm. Thus, cues from fish appear to be more influential to daphnid growth than cues from epiphyton.

Our experiment in Lake Stigsholm allowed us to address the additional impact of submerged macrophytes on *Daphnia* growth. The largest suppression of daphnid growth occurred for daphnids housed in filtered water from the wholly vegetated lake, which has a low abundance of fish (S. Berg unpubl.). The lake water used came from within permanent, dense stands of *Elodea canadensis* that blanketed the lake and reached to the water's surface. If we can apply the low impact of epiphytes from Lake Torup to Lake Stigsholm, then it seems that chemical cues from *Elodea* played the most important role in reducing daphnid growth in Lake Stigsholm. However, we cannot fully

exclude the possibility that toxic strains of *Microcystis* (Christoffersen unpubl.) may have played a role in Lake Stigsholm, although phytoplankton chlorophyll *a* concentrations were low (Table 1).

Our laboratory experiment demonstrated that chemicals from a widespread macrophyte (Nichols and Shaw 1986) and common fish suppressed daphnid growth. Our laboratory results agree with our field experiments. In addition to growth, these chemical cues also altered *Daphnia* life-history traits. Multiple studies demonstrate that exposure to fish chemical cues results in a smaller size at first reproduction (Stibor 1992, Engelmayer 1995) but none document the impact of macrophyte chemicals. Contrary to our predictions, daphnids in media containing *Elodea* took longer to reach the size at which daphnids in the control media reproduced. However, as expected, daphnids in media containing *Elodea* possessed fewer eggs. These life-history alterations could detrimentally impact *Daphnia* undergoing DHM as a predator-avoidance strategy (Lauridsen and Buenk 1996). Longer reproduction time translates into increased predation exposure, and fewer eggs per female slows population increase (Threlkeld 1987).

Based on other studies, we expected daphnids to be smallest and reproduce earliest when exposed to fish chemicals (Weider and Pijanowska 1993, Reede 1995). However, daphnids grown in media containing roach chemical cues did not significantly differ from daphnids in control media in either age or size at first reproduction, although a tendency to be reduced in size at first reproduction was observed. However, they did possess fewer eggs as expected. Egg number and size at first reproduction of daphnids held in media containing roach also did not statistically differ from daphnids kept in media with both roach and *Elodea*. Thus, the effect of roach chemicals is puzzling. Daphnids held in media with *Elodea* and roach responded differently than daphnids held only with *Elodea*, indicating that chemical cues from roach interacted with chemical cues from *Elodea*.

The individual responses alone of *Daphnia* to either roach or *Elodea* did not predict how *Daphnia* responded to the combined influence of multiple chemical cues. As daphnids exposed to fish chemicals would likely already reproduce earlier at a smaller size (Reede 1995), we did not expect to find an additional negative impact of macrophyte chemicals. However, daphnids housed in media containing both *Elodea* and roach showed the shortest reproduction times at nearly the smallest size. In contrast to a greater decrease in reproductive age for daphnids exposed to both cues versus single cues, other life-history traits did not show predictable trends between single and multiple cues. Size at maturity was large for daphnids exposed to *Elodea* and small for daphnids exposed to roach. Compared with daphnids only exposed to roach cues, addition of *Elodea* cues neither significantly increased nor de-

creased daphnid size at maturity as one might predict. Additionally, egg number did not differ between daphnids exposed to *Elodea*, roach or both.

The non-additivity of these responses to *Elodea* and roach cues suggests natural combinations of chemical cues produce significant responses that are not predictable from combining experiments that only investigate single factors (Morin 1995). These results could be particularly relevant for daphnids undergoing diel horizontal migration in shallow lakes in which they are likely to encounter multiple cues. Daphnids undergoing DVM primarily stay in the relatively homogeneous, pelagic zone and likely receive chemical cues mainly from visually foraging planktivores (O'Brien and Wright 1985). Forced to seek alternative refuge in littoral zones, daphnids could encounter additional chemical influences from macrophytes.

Our results indicate that costs, such as delayed reproduction and fewer eggs, could accompany prolonged association with macrophytes. These costs could offer one explanation for observed chemical avoidance of macrophytes by daphnids (Hasler and Jones 1949, Lauridsen and Lodge 1996). We might speculate that other submerged macrophytes, such as *Myriophyllum exalbescens*, which daphnids have been shown to avoid (Lauridsen and Lodge 1996), could also influence growth or life history traits but this has not been tested. Submerged macrophytes, such as *E. canadensis*, inhibit phytoplankton growth but also serve as an important habitat for other invertebrates (Nichols and Shaw 1986, Kornijów and Kairesalo 1994, Diehl and Kornijów 1998). Increased grazing of phytoplankton and epiphyton (Horton et al. 1979) by large cladocerans is likely to positively impact submerged macrophytes (Stansfield et al. 1997, Jeppesen 1998). Thus, the negative influence of macrophyte chemical cues on *Daphnia* is perplexing.

Other studies have shown indirect effects of fish chemical cues on behavior (Huang and Sih 1991) and life-history traits (Machaček 1991). One possibility is that chemicals exuded by macrophytes are intended to inhibit algae (Phillips et al. 1978) and daphnids experience negative effects indirectly. Chemical cues intended to deter other invertebrate grazers (Ostrofsky and Zettler 1986, Pip 1992) may also directly influence daphnids. Whether by direct or indirect effects, chemical cues from macrophytes are likely to affect daphnids venturing into littoral zones.

Under low predation pressure or with alternative refuge, there seems little reason for daphnids to migrate into littoral zones that often contain less phytoplankton (Phillips et al. 1978, Schriver et al. 1995). This is particularly relevant if the phytoplankton is of lower quality than in the pelagic (Kairesalo 1980, Smiley and Tessier 1998), although opinion varies about food quality in vegetated zones (Søndergaard and Moss 1998, Van den Berg et al. 1998). However, under high predation pressure from fish (Jeppesen et al. 1998), daphnids

may need the protection offered by macrophytes. Habitat switches which minimize μ/g (rates of mortality/rates of growth) for daphnids should be favored (Werner and Gilliam 1984). As shown in our studies, daphnids may suffer reduced growth or alter their life-history strategies among littoral zone chemicals. However, these costs must be balanced against the benefits of reduced predation to assess whether horizontal migration is advantageous.

References

Brooks, J. L. and Dodson, S. I. 1965. Predation, body size and composition of plankton. – Science 150: 28–35.

Canfield, D. E. Jr., Shireman, J. V., Colle, D. E. et al. 1984. Prediction of chlorophyll *a* concentrations in Florida lakes: importance of aquatic macrophytes. – Can. J. Fish. Aquat. Sci. 41: 497–501.

Carpenter, S. R. and Kitchell, J. F. (eds) 1993. The trophic cascade in lakes. – Cambridge Univ. Press, Cambridge.

de Bernardi, R. and Peters, R. H. 1987. Why *Daphnia*? – Mem. Ist. Ital. Idrobiol. 45: 1–9.

DeMeester, L. 1993. Genotype, fish-mediated chemicals, and phototactic behavior in *Daphnia magna*. – Ecology 74: 1467–1474.

Dicke, M. and Sabelis, M. W. 1988. Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? – Funct. Ecol. 2: 131–139.

Diehl, S. and Kornijów, R. 1998. Influence of submerged macrophytes on trophic interactions among fish and macroinvertebrates. – In: Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. and Christoffersen, K. (eds), The structuring role of submerged macrophytes in lakes. Springer-Verlag, New York, pp. 24–46.

Dodson, S. I. 1988a. The ecological role of chemical stimuli for the zooplankton: predator-avoidance behavior in *Daphnia*. – Limnol. Oceanogr. 33: 1431–1439.

Dodson, S. I. 1988b. The ecological role of chemical stimuli for the zooplankton: predator-induced morphology in *Daphnia*. – Oecologia 78: 361–367.

Edmondson, W. T. and Litt, A. H. 1982. *Daphnia* in Lake Washington. – Limnol. Oceanogr. 27: 272–293.

Engelmayer, A. 1995. Effects of predator-released chemicals on some life history parameters of *Daphnia pulex*. – Hydrobiologia 307: 203–206.

Gross, E. M., Wolk, P. C. and Jüttner, F. 1991. Fischerellin, a new allelochemical from the freshwater cyanobacterium *Fischerella muscicola*. – J. Phycol. 27: 686–692.

Hasler, A. D. and Jones, E. 1949. Demonstration of the antagonistic action of large aquatic plants on algae and rotifers. – Ecology 30: 359–364.

Hessen, D. O. and Van Donk, E. 1993. Morphological changes in *Scenedesmus* induced by substances released by *Daphnia*. – Arch. Hydrobiol. 127: 129–140.

Horton, P. A., Rowan, M., Webster, K. E. and Peters, R. H. 1979. Browsing and grazing by cladoceran filter feeders. – Can. J. Zool. 57: 206–212.

Hrbaček, J., Dvorakova, M., Korinek, M. and Prochazkova, L. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. – Verh. Int. Ver. Limnol. 14: 192–195.

Huang, C. and Sih, A. 1991. Experimental studies on direct and indirect interactions in a three trophic-level stream system. – Oecologia 85: 530–536.

Jasser, I. 1995. The influence of macrophytes on a phytoplankton community in experimental conditions. – Hydrobiologia 306: 21–32.

Jeppesen, E. 1998. The ecology of shallow lakes – biological interactions in the pelagial. – DSc. Ministry of Environment and Energy, National Environmental Research Institute. NERI, Report 247.

Jeppesen, E., Jensen, J. P., Søndergaard, M. and Lauridsen, T. L. 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. – Hydrobiologia 342/343: 151–164.

Jeppesen, E., Lauridsen, T. L., Kairesalo, T. and Perrow, M. R. 1998. Impact of submerged macrophytes on fish-zooplankton interactions in lakes. – In: Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. and Christoffersen, K. (eds), The structuring role of submerged macrophytes in lakes. Springer-Verlag, New York, pp. 91–114.

Jespersen, A. M. and Christoffersen, K. 1987. Measurements of chlorophyll *a* from phytoplankton using ethanol as extraction solvent. – Arch. Hydrobiol. 109: 445–454.

Kairesalo, T. 1980. Diurnal fluctuations within a littoral plankton community in oligotrophic Lake Pääjärvi southern Finland. – Freshwater Biol. 10: 533–537.

Kats, L. B. and Dill, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. – Ecoscience 5: 361–394.

Kleiven, O. T., Larsson, P. and Hobæk, A. 1996. Direct distributional response in *Daphnia pulex* to a predator kairomone. – J. Plankton Res. 18: 1341–1348.

Kornijów, R. and Kairesalo, T. 1994. *Elodea canadensis* sustains rich environment for macroinvertebrates. – Verh. Int. Ver. Limnol. 25: 4098–4111.

Kvam, O. V. and Kleiven, O. T. 1995. Diel horizontal migration and swarm formation in *Daphnia* in response to *Chaoborus*. – Hydrobiologia 307: 177–184.

Lampert, W. 1993. Ultimate causes of diel vertical migration of zooplankton: new evidence for the predator-avoidance hypothesis. – Arch. Hydrobiol. Beih. Egeb. Limnol. 39: 79–88.

Lampert, W., Rothhaupt, K. O. and Von Elert, E. 1994. Chemical induction of colony formation in a green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). – Limnol. Oceanogr. 39: 1543–1550.

Larsson, P. and Dodson, S. I. 1993. Invited review: chemical communication in planktonic animals. – Arch. Hydrobiol. 129: 129–155.

Lauridsen, T. L. and Buenk, I. 1996. Diel changes in the horizontal distribution of zooplankton in the littoral zone of two shallow eutrophic lakes. – Arch. Hydrobiol. 137: 167–176.

Lauridsen, T. L. and Lodge, D. M. 1996. Avoidance by *Daphnia magna* of fish and macrophytes: chemical cues and predator-mediated use of macrophyte habitat. – Limnol. Oceanogr. 41: 794–798.

Lauridsen, T. L., Pedersen, L. J., Jeppesen, E. and Søndergaard, M. 1996. The importance of macrophyte bed size for cladoceran composition and horizontal migration in a shallow lake. – J. Plankton. Res. 18: 2283–2294.

Lauridsen, T. L., Jeppesen, E., Søndergaard, M. and Lodge, D. M. 1998. Horizontal migration of zooplankton: predator-mediated use of macrophyte habitat. – In: Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. and Christoffersen, K. (eds), The structuring role of submerged macrophytes in lakes. Springer-Verlag, New York, pp. 233–239.

Lauridsen, T. L., Jeppesen, E., Mitchell, S. F., Lodge, D. M. and Burks, R. L. 1999. Horizontal distribution of zooplankton in lakes with contrasting fish densities and nutrient levels. – Hydrobiologia.

Macháček, J. 1991. Indirect effect of planktivorous fish on the growth and reproduction of *Daphnia galeata*. – Hydrobiologia 225: 193–197.

Morin, P. J. 1995. Functional redundancy, non-additive interactions, and supply-side dynamics in experimental pond communities. – Ecology 76: 133–149.

Moss, B., Kornijów, R. and Measey, G. J. 1998. The effects of nymphaeid (*Nuphar lutea*) density and predation by perch (*Perca fluviatilis*) on the zooplankton community in a shallow lake. – Freshwat. Biol. 39: 689–697.

Nichols, S. A. and Shaw, B. H. 1986. Ecological life histories of the three aquatic nuisance plants: *Myriophyllum spicatum*, *Potamogeton crispus* and *Elodea canadensis*. – Hydrobiologia 131: 3–21.

O'Brien, W. J. and Wright, D. I. 1985. Potential limits on the daytime planktivorous feeding depth of white crappie. – Verh. Int. Ver. Limnol. 22: 2527–2533.

Ostrofsky, M. L. and Zettler, E. R. 1986. Chemical defences in aquatic plants. – J. Ecol. 74: 279–287.

Pennak, R. W. 1973. Some evidence for aquatic macrophytes as repellents for a limnetic species of *Daphnia*. – Int. Rev. Gesamten Hydrobiol. 58: 569–576.

Perrow, M. R., Meijer, M. L., Dawidowicz, P. and Coops, H. 1997. Biomanipulation in shallow lakes: state of the art. – Hydrobiologia 342/343: 355–365.

Phillips, G. L., Eminson, D. F. and Moss, B. 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. – Aquat. Bot. 4: 103–126.

Pijanowska, J. 1997. Alarm signals in *Daphnia*? – Oecologia 112: 12–16.

Pip, E. 1992. Phenolic compounds in macrophytes from the Lower Nelson River system, Canada. – Aquat. Bot. 42: 273–279.

Reede, T. 1995. Life history shifts in response to different levels of fish kairomones in *Daphnia*. – J. Plankton Res. 17: 1661–1667.

Reede, T. and Ringelberg, J. 1995. The influence of a fish exudate on two clones of the hybrid *Daphnia galeata × hyalina*. – Hydrobiologia 307: 207–212.

Sakwińska, O. 1998. Plasticity of *Daphnia magna* life history traits in response to temperature and information about a predator. – Freshwat. Biol. 39: 681–687.

Schriver, P., Bøgstrand, J., Jeppesen, E. and Søndergaard, M. 1995. Impact of submerged macrophytes on fish-zooplankton-phytoplankton interactions: large-scale enclosure experiments in a shallow eutrophic lake. – Freshwat. Biol. 33: 255–270.

Smiley, E. A. and Tessier, A. J. 1998. Environmental gradients and the horizontal distribution of microcrustaceans in lakes. – Freshwat. Biol. 39: 397–409.

Søndergaard, M. and Moss, B. 1998. Impact of submerged macrophytes on phytoplankton in shallow freshwater lakes. – In: Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. and Christoffersen, K. (eds), The structuring role of submerged macrophytes in lakes, Springer-Verlag, New York, pp. 115–132.

Stansfield, J. H., Perrow, M. R., Tench, L. D. et al. 1997. Submerged macrophytes as refuge for grazing Cladocera against fish predation: observations on seasonal changes in relation to macrophyte cover and predation pressure. – Hydrobiologia 342/343: 229–240.

Stibor, H. 1992. Predator induced life-history shifts in a freshwater cladoceran. – Oecologia 92: 162–165.

Threlkeld, S. T. 1987. *Daphnia* life history strategies and resource allocation patterns. – Mem. Ist. Ital. Idrobiol. 353-366.

Timms, R. M. and Moss, B. 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. – Limnol. Oceanogr. 29: 472–486.

Van den Berg, M. S., Coops, H., Meijer, M.-L. et al. 1998. Clear water associated with dense *Chara* vegetation in the shallow and turbid Lake Veluwemeer, The Netherlands. – In: Jeppesen, E., Søndergaard, Ma., Søndergaard, Mo. and Christoffersen, K. (eds), The structuring role of submerged macrophytes in lakes. Springer-Verlag, New York, pp. 339–352.

Vanni, M. J. 1987. Indirect effect of predators on age-structured prey populations: planktivorous fish and zooplankton. – In: Kerfoot, W. C. and Sih, A. (eds), Predation: direct and indirect impacts on aquatic communities. Univ. Press of New Hampshire, Durham, NH, pp. 149–160.

Von Elert, E. and Loose, C. J. 1996. Predator-induced diel vertical migration in *Daphnia*: enrichment and preliminary chemical characterization of a kairomone exuded by fish. – J. Chem. Ecol. 22: 885–895.

Watt, P. J. and Young, S. 1994. Effect of predator chemical cues on behaviour in both horizontal and vertical planes. – Anim. Behav. 48: 861–869.

Weider, L. J. and Pijanowska, J. 1993. Plasticity of *Daphnia* life histories in response to chemical cues from predators. – Oikos 67: 385–392.

Werner, E. E. and Gilliam, J. F. 1984. The ontogenetic niche and species interactions in size-structured populations. – Annu. Rev. Ecol. Syst. 15: 393–425.