

The lethal and sublethal effects of the aquatic macrophyte *Myriophyllum spicatum* on Baltic littoral planktivores

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Abstract

Macrophyte architecture can structure predator–prey interactions, but it is the chemicals within the plant that may actually be lethal. We conducted aquarium experiments to study the effects of common aquatic macrophytes (*Myriophyllum spicatum*, *Myriophyllum sibiricum*, and *Chara tomentosa*) and a predator (perch, *Perca fluviatilis*) on the survival, habitat choice, swimming, and feeding activities of Baltic littoral planktivores, mysids *Neomysis integer* and *Praunus flexuosus*, and three-spined stickleback (*Gasterosteus aculeatus*) larvae. Chemicals excreted by *M. spicatum* in a dense patch caused high mortality (73% to 89%) in both mysid species but not in sticklebacks, whereas *M. sibiricum* and *C. tomentosa* had no lethal effects. In lower stem densities stickleback larvae and *N. integer* avoided *M. spicatum* even in the presence of predator signals, and *M. spicatum* lowered the swimming and feeding activities of stickleback larvae. Only *P. flexuosus* did not avoid *M. spicatum* vegetation. Areas occupied by *M. spicatum* seem to be highly unsuitable habitats for littoral mysids and three-spined stickleback larvae. Because *M. spicatum* is a dominant macrophyte in the study area and eutrophication further increases its abundance, it may strongly influence the occurrence and distribution of mysids and fish larvae in the littoral ecosystems of the Baltic Sea.

Complex macrophytes present foraging challenges to many fishes (e.g., Moss et al. 1998); therefore, littoral organisms can avoid predation by hiding among aquatic macrophyte vegetation (Burks et al. 2001). Various littoral planktivores such as mysid shrimps (Lindén et al. 2003) and three-spined sticklebacks (Jakobsen et al. 1988; Lehtiniemi in press) hide in the vegetation in the presence of predators. Other predator avoidance strategies include reductions in motility and feeding rate (Milinski and Heller 1978; Ejdung

1998), which all aim to minimize the conspicuousness of the prey to predators. Early life stages of fish are especially vulnerable to predation (Pedersen 1997), and seeking refuge among vegetation is an essential part of their predation avoidance (e.g., Romare and Hansson 2003). However, not all macrophytes may be suitable hiding places for aquatic animals due to their structural or chemical characteristics (Flynn and Ritz 1999; Burks et al. 2000). In fact, early studies (Matheson 1930; Hasler and Jones 1949) documented the lethal impact of certain macrophytes to many aquatic organisms, such as phytoplankton, rotifers, and mosquito larvae. Therefore, animals may be facing a trade-off situation (reviewed in Lima and Dill 1990) between avoiding predation and avoiding a harmful refuge.

The milfoils *Myriophyllum* spp. (Haloragaceae) and the green algae *Chara* spp. (Characeae) have negative effects on other aquatic organisms. Several *Myriophyllum* species inhibit the growth of phytoplankton, especially cyanobacteria (reviewed in Gross 2003), and repel cladocerans (Pennak 1973; Lauridsen and Lodge 1996). Potentially the most

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Acknowledgments

We thank R. Munsterhjelm for his help in the determination of macrophytes and T. Hakala, A. Hirvonen, S. Londesborough, and T. Meriläinen for their help in the field. The Tvärminne Zoological Station, University of Helsinki, provided field equipment and laboratory facilities. M. Karjalainen, J.-P. Pääkkönen, M. Viitasalo, and two anonymous reviewers commented on an earlier version of the manuscript. This study was financed by the Walter and Andrée de Nottbeck Foundation and the Academy of Finland.

harmful species in the genus is *Myriophyllum spicatum*, which has negative effects on phytoplankton (reviewed in Gross 2003), other macrophytes (Agami and Waisel 1985), and animals, such as mosquitoes and midges (Dhillon et al. 1982; Johnson and Mulla 1983). Similarly, *Chara* spp. inhibit planktonic bacteria and algae (e.g., Bankova et al. 2001; Berger and Schagerl 2003) and are repellent or toxic to insects, especially mosquitoes (e.g., Matheson 1930; Jacobsen and Pedersen 1983).

The aim of the present study was to investigate the effects of three common aquatic macrophyte species, *Myriophyllum spicatum*, *Myriophyllum sibiricum*, and *Chara tomentosa*, on the survival, predator avoidance behavior, and feeding of common littoral planktivores, i.e., mysids *Neomysis integer* (Leach) and *Praunus flexuosus* (Müller) and three-spined stickleback (*Gasterosteus aculeatus* L.) larvae. *Myriophyllum* spp. and *Chara* spp. are abundant macrophytes at many coastal areas of the northern Baltic Sea and provide potential hiding places for the studied planktivores. *M. spicatum* and *M. sibiricum* are very similar in their architecture but slightly more densely branching than *C. tomentosa*. All three species form similar, complex habitats. Littoral mysids are an important link between several trophic levels: they are omnivorous, feeding on phytoplankton, zooplankton, and detritus (Mauchline 1980), and they are also important prey for a number of fish species, such as perch (*Perca fluviatilis*) and Baltic herring (*Clupea harengus membras*) (Thiel 1996). *N. integer* forms large swarms in open littoral areas and migrates horizontally between seasons (M. Kauppila unpubl. data). *P. flexuosus* is strongly associated with aquatic macrophytes, such as the bladder wrack *Fucus vesiculosus*, and produces usually only one generation per year in the study area, whereas *N. integer* produces two (M. Kauppila unpubl. data). Three-spined stickleback, in turn, is the most common fish species in the study area (Sundell 1994) and prey for a wide regime of piscivores (Reimchen 1994). We provided the mysids and the stickleback larvae both chemical and visual predator signals and hypothesized that they would adjust their habitat choice and swimming and feeding activity according to predation risk and suitability of the macrophyte habitat as a refuge.

Materials and methods

We performed two kinds of laboratory experiments. First, *N. integer*, *P. flexuosus*, and three-spined stickleback larvae were incubated with *M. spicatum*, *M. sibiricum*, and *C. tomentosa* to find out how these macrophytes influence the survival of planktivores. According to the results of these mortality experiments, we chose *M. spicatum*, the most harmful macrophyte, for the behavior experiments. Second, we studied the habitat choice and swimming activity of mysids and three-spined stickleback larvae in the presence of *M. spicatum*, artificial vegetation, and predator signals from perch by videofilming. The feeding activity of stickleback larvae was also determined.

Sampling—We conducted the experiments at Tvärminne Zoological Station (Univ. Helsinki), southwest coast of Finland, northern Baltic Sea. *M. spicatum*, *M. sibiricum*, and *C.*

tomentosa were collected by hand and with a rake from shallow bays nearby. We collected adult female mysids (*N. integer*, mean length 16 mm and dry weight 4.9 mg; and *P. flexuosus*, mean length 24 mm and dry weight 14.0 mg), three-spined stickleback (*G. aculeatus*) larvae (mean length 9.1 mm and dry weight 3.4 mg), and adult (ca. 15 cm) European perch (*Perca fluviatilis* L.) from the littoral zone and transported them to the laboratory in ambient seawater. Mysids and perch acclimated to the laboratory conditions for at least 8 h, while sticklebacks took 5 d. The stickleback larvae were provided with natural zooplankton (>100 μ m) three times a day after yolk sac absorption. The mysids were also fed with natural zooplankton. The perch were fed with littoral mysids before the mysid experiments and stickleback larvae before the stickleback experiments. We returned all perch to the sea after the experiments. We used perch in the experiments because it is a common piscivore in the area (Sundell 1994), inhabiting shallow bays and littoral zones in which mysids and three-spined sticklebacks also dwell.

Mortality experiments—We conducted the mortality experiments in rectangular 2.2-liter aquaria (width 15.1 cm \times depth 7.2 cm \times height 20 cm) with *M. spicatum*, *M. sibiricum*, *C. tomentosa*, or without macrophytes at 16°C in normal indoor light conditions. With *N. integer* we conducted experiments with all three macrophyte species and without macrophytes. With *P. flexuosus* we conducted experiments with *M. spicatum*, *M. sibiricum*, and without macrophytes, and with three-spined stickleback larvae we conducted experiments with *M. spicatum* and without macrophytes. The stems of *M. spicatum*, *M. sibiricum*, and *C. tomentosa* were cut to fit the height of the aquaria and placed through holes in metal plates that were positioned on the bottom of the aquaria in 0.2- μ m filtered seawater. There were 26 stems in each aquarium, except the control aquaria, which had no macrophytes. We added one mysid or fish larva to each aquarium using a small sieve. The experiments lasted for 3 h, after which we determined the survival of the animals. We tested the data for statistical significance with Fisher's exact test. The mysids and larvae, which were instantly killed by decapitation, were measured to the nearest millimeter and dried at 60°C for ca. 12 h, after which their dry weight was measured with a Mettler AE 100 balance.

Behavior experiments with mysids—In the behavior experiments, we conducted the artificial vegetation treatments (green plastic cords, placed through metal plates as described above) in similar aquaria (width 15.1 cm \times depth 7.2 cm \times height 20 cm) and conditions as described previously. To prevent mortality of mysids, the *M. spicatum* treatments were, however, conducted in a larger aquarium (20 cm \times 20 cm), in which the mysids were placed in a compartment (separated with a 200- μ m net), which was approximately of the same size and shape as the aquaria where artificial vegetation treatments were conducted (Fig. 1). In both macrophyte treatments the metal plate (with 13 stems) covered one half of the aquarium bottom, thus leaving the other half of the aquarium vegetation free. In the predator signal treatments we filled the aquarium with water from the perch container and placed an aquarium containing a perch

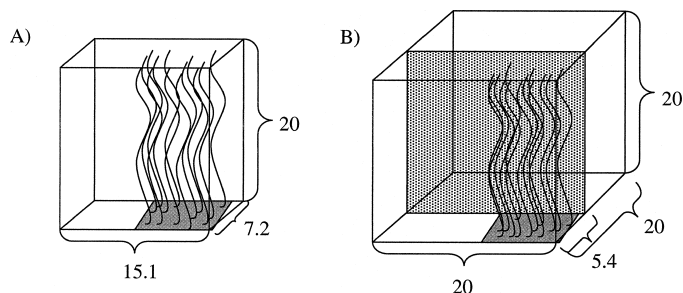


Fig. 1. Design of experimental aquaria used in (A) behavior experiments with mysids in artificial vegetation treatments and with sticklebacks and in (B) behavior experiments with mysids in *M. spicatum* treatments. Dimensions are indicated as centimeters (cm).

next to the experimental aquarium so that the perch was clearly visible to the mysid but real predation by the perch was prevented. Hence, both chemical and visual predator signals (which are required to produce a behavioral response in littoral mysids; Lindén et al. 2003) were present. The control aquaria were filled with 0.2- μm filtered seawater, and no visual predator signal was added. We provided the mysids with natural zooplankton ($>100\ \mu\text{m}$) during all behavior experiments to ensure normal activity.

We placed one mysid at a time into the aquarium using a small sieve and studied its behavior by videofilming. We used two similar video cameras simultaneously to conduct the experiments in as short a time as possible. The mysid was placed into the aquarium ca. 2 min before the beginning of the filming. Each filming lasted for 15 min, after which we changed the mysid, water, macrophytes, and perch and started the following video sessions. There were 10 replicates for each mysid species in each vegetation type in both predator treatments. After the experiments, the mysids were measured to the nearest millimeter and dried at 60°C for ca. 12 h, after which their dry weight was measured.

We analyzed the videotapes to observe the behavior of mysids. We recorded the duration of swimming versus immobility and the time spent in the vegetation versus in the open water and calculated them as percentages of total time. We could not reliably distinguish the feeding activity of mysids from the tapes, and therefore it is not presented. The data did not fulfill the assumptions of parametric tests and were therefore tested for statistical significance with nonparametric Mann–Whitney *U*-test.

Behavior experiments with three-spined sticklebacks—We conducted the experiments in similar aquaria (width 15.1 cm \times depth 7.2 cm \times height 20 cm) and conditions as described previously. There were two vegetation treatments (*M. spicatum* and artificial vegetation, placed through metal plates as described previously) and three predator treatments (no signals, chemical signal, and combined chemical and visual signal). In the chemical predator signal treatment we added 0.2 liters of water from the perch tank into the aquarium. This amount is sufficient to produce a response in stickleback larvae (Lehtiniemi in press). The rest of the water in the aquarium was 0.2- μm filtered seawater. The combined chemical and visual predator signal treatment consisted of

the same chemical signal with a perch placed into an aquarium next to the experimental aquarium. The control aquaria contained 0.2- μm filtered seawater and artificial vegetation or *M. spicatum*. All treatments were replicated six times and performed in randomized order. In order to determine the feeding rate of the larvae, we provided them with natural zooplankton ($>100\ \mu\text{m}$) during the experiments. Prior to the experiments zooplankton was held in a 30-liter tank with aeration. Before the addition of prey to the experimental aquarium we mixed the zooplankton tank thoroughly and added an equal amount of zooplankton into the aquaria. We adjusted the zooplankton density in the experimental aquarium to a level above natural density (>20 copepods L^{-1} , cf. Viitasalo et al. 1995) to prevent prey depletion and, hence, ensure continuous feeding of the larvae.

We placed one three-spined stickleback larva at a time into the open water side of the aquarium using a small sieve. We turned the video on after checking that the larva was in the open water and could easily be seen on the videotape. Each filming lasted for 20 min, after which we changed the larva, water, macrophytes, and perch and started the following video sessions. As with mysids, we used two similar video cameras simultaneously. After the experiments, 10 fish were instantly killed by decapitation, measured to the nearest millimeter (total length with head), and then dried at 60°C for ca. 12 h, after which their dry weight was measured. We analyzed the videotapes as described previously. In addition, we counted the number of attacks on prey. An attack was determined as a rush forward to capture a prey (cf. Viitasalo et al. 1998). We did not measure the actual prey consumption because we could not determine the escape success of the zooplankton prey from the tapes.

We tested the habitat choice and swimming activity data for statistical significance with two-way analysis of variance (ANOVA), followed by Tukey honestly significant difference (HSD) multiple comparisons, after $\log(x + 1)$ transformation on the habitat choice data. The attack rate data did not fulfill the assumptions of parametric tests and was thus tested for statistical significance with Kruskal–Wallis nonparametric one-way analysis of variance, followed by Tukey HSD multiple comparisons.

Results

Mortality experiments—In the mortality experiments, *M. spicatum* was acutely lethal to mysids. Eight out of 9 *N. integer* and 8 out of 11 *P. flexuosus* individuals died within 3 h in the aquaria with *M. spicatum*, which correspond to 89% and 73% mortality, respectively. The surviving mysids were also in weak condition, turning from transparent to white. In the control (filtered sea water) *M. sibiricum* and *C. tomentosa* treatments no mortality was observed. There was a statistically significant difference between *M. spicatum* treatment and the control, *C. tomentosa* and *M. sibiricum* treatments (Fisher's exact test, $p < 0.001$), but not between the two mysid species in the *M. spicatum* treatment (Fisher's exact test, $p > 0.05$).

Three-spined stickleback larvae experienced no mortality in the experiments, neither in control nor in *M. spicatum* treatment.

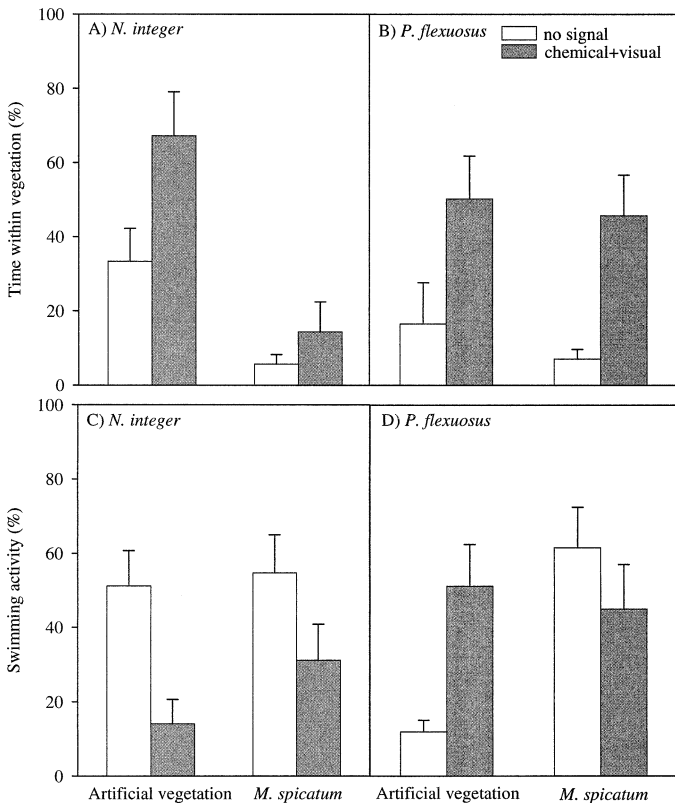


Fig. 2. (A, B) Time spent within vegetation, and (C, D) swimming activity (%; mean + SE) of *N. integer* and *P. flexuosus* in the presence of artificial vegetation and *M. spicatum* in no signal and combined chemical and visual predator signal treatments ($n = 10$). The data on artificial vegetation have been published previously in Lindén et al. (2003).

Behavior experiments with mysids—In the behavior experiments with mysids, both mysid species responded to the presence of predator signals, whereas only *N. integer* responded to the presence of *M. spicatum*. *N. integer* spent significantly less time within *M. spicatum* vegetation than within artificial vegetation (Mann–Whitney, $U = 355.5$, $df = 1$, $p < 0.001$), whereas the predator signals did not have a significant effect on their time spent within vegetation (Mann–Whitney, $U = 140.0$, $df = 1$, $p = 0.107$) (Fig. 2A). In contrast, *P. flexuosus* spent significantly more time within the vegetation in the presence of predator signals (Mann–Whitney, $U = 46.0$, $df = 1$, $p < 0.001$), whereas their time spent within *M. spicatum* did not differ from their time spent within artificial vegetation (Mann–Whitney, $U = 198.0$, $df = 1$, $p = 0.956$) (Fig. 2B).

The presence of *M. spicatum* did not affect the swimming activity of the mysids (Mann–Whitney for *N. integer*, $U = 148.0$, $df = 1$, $p = 0.159$; for *P. flexuosus*, $U = 135.5$, $df = 1$, $p = 0.081$) (Fig. 2C,D). The predator signals decreased the swimming activity of *N. integer* (Mann–Whitney, $U = 307.0$, $df = 1$, $p = 0.004$), whereas no clear pattern was found with *P. flexuosus* (Mann–Whitney, $U = 160.0$, $df = 1$, $p = 0.279$).

Behavior experiments with three-spined sticklebacks—Both macrophytes and predator signals had significant ef-

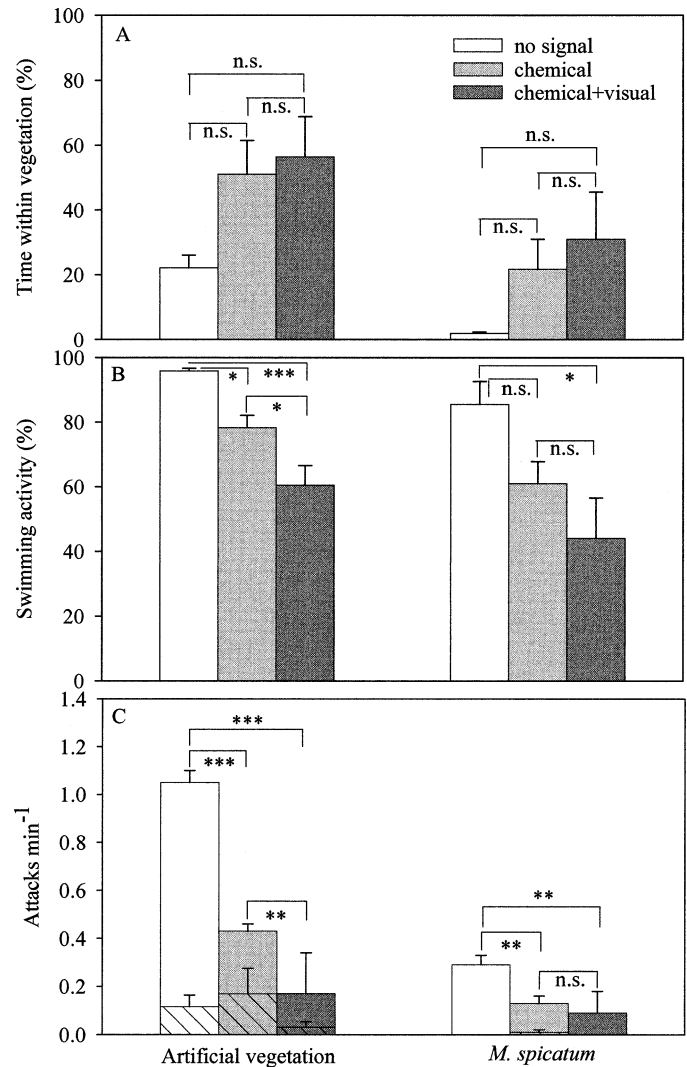


Fig. 3. (A) Time spent within vegetation (%), (B) swimming activity (%), and (C) attack rate (attacks min^{-1}) (mean + SE) of *G. aculeatus* in the presence of artificial vegetation and *M. spicatum* in no signal, chemical predator signal, and combined chemical and visual predator signal treatments ($n = 6$). Attacks made within vegetation are shown as striped bars. Asterisks denote the results of Tukey HSD multiple comparisons, where $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

fects on the behavior of the three-spined stickleback larvae. The presence of *M. spicatum* decreased the time spent within vegetation (two-way ANOVA, $F_{1,30} = 20.644$, $p < 0.001$) (Fig. 3A), swimming activity (two-way ANOVA, $F_{1,30} = 6.364$, $p = 0.017$) (Fig. 3B), and attack rate (Kruskal–Wallis, $U = 264.500$, $df = 1$, $p = 0.001$) (Fig. 3C) of sticklebacks compared with artificial vegetation. Significantly fewer attacks were made within vegetation than in open water (Kruskal–Wallis, $U = 197.000$, $df = 1$, $p < 0.001$) and within *M. spicatum* vegetation than within artificial vegetation (Kruskal–Wallis, $U = 237.000$, $df = 1$, $p = 0.003$). When predator signals were present, the larvae spent significantly more time within the vegetation (two-way ANOVA, $F_{2,30} = 5.138$, $p = 0.012$) and reduced significantly their swimming activity

(two-way ANOVA, $F_{2,30} = 14.470$, $p < 0.001$) as well as attack rate (Kruskal–Wallis, $K-W = 14.841$, $df = 2$, $p = 0.001$). The predator signals and *M. spicatum* had no significant combined effects on the habitat choice (two-way ANOVA, $F_{2,30} = 0.469$, $p = 0.630$) or swimming activity ($F_{2,30} = 0.143$, $p = 0.867$). The combined chemical and visual predator signal had a stronger impact on larval behaviors than the chemical signal alone, although the difference between these two treatments was not statistically significant in all cases (Tukey, $p > 0.05$) (Fig. 3).

Discussion

Myriophyllum spicatum caused high mortality in both mysid species studied, *N. integer* and *P. flexuosus*. This is the first time a larger aquatic invertebrate has been shown to be killed by submerged macrophytes. Also, although not experiencing any mortality, the three-spined stickleback larvae clearly avoided *M. spicatum*, and almost no attacks on zooplankton took place within *M. spicatum* vegetation. The repellent effect of *M. spicatum* on the larvae was especially conspicuous when predator signals were not present.

We suggest that the mortality and other adverse effects of *M. spicatum* were caused by chemicals excreted by this macrophyte species. *M. spicatum* produces and releases polyphenols that have inhibitory effects on phytoplankton and cyanobacteria (reviewed in Gross 2003). The main inhibitory compound, hydrolysable polyphenol tellimagrandin II, inactivates extracellular enzymes of algae that make additional nutrients available for uptake and inhibits the activity of photosystem II by interfering with the electron transfer chain (reviewed in Gross 2003). According to Nakai et al. (2001), the autoxidation products of polyphenols may also fatally damage the target cells. In our mortality experiments, the mysids were immobilized in less than a minute after introduction to the *M. spicatum* vegetated aquaria, which suggests that the toxins rapidly kill or paralyze the animals. The incapability to produce a respiration current may also suffocate the mysids. However, the mechanisms behind the adverse effects of *M. spicatum* to animals have not been studied.

Phenolic substances serve a variety of functions for plants and are well known for their toxic effects on man and animals, as well as microbial organisms (Singleton and Kratzer 1969; Levin 1971). Possible mechanisms behind the toxicity and repellence to both invertebrates and vertebrates are numerous, such as membrane damaging activity or interference with catecholamine metabolism and other neural control mechanisms (reviewed by Singleton and Kratzer 1969). A chemical isolated from *Chara globularis* (4-methylthio-1,2-dithiolane, also named charatoxin, Anthoni et al. 1980) blocks the cholinergic receptors and thus inhibits neural functions, and kills houseflies (*Musca domestica*) (Jacobsen and Pedersen 1983). Such compounds have, however, not been identified from *M. spicatum*. Furthermore, in our experiments *M. sibiricum*, albeit belonging to the same genus as *M. spicatum* and having a high phenolic acid content (Spencer and Ksander 1999), induced no mortality in mysids. Therefore the mechanism that caused the high mortality in mysids remains unknown.

The ultimate reason for the production of repellent chemicals in aquatic macrophytes may be herbivore deterrence (Dhillon et al. 1982; Cronin and Hay 1996a), gaining competitive advantage over other autotrophs (Gross 2003) or both. If the toxicity is primarily an inducible defense against herbivores, tissue damage may facilitate the production and excretion of the chemicals (Levin 1971; Cronin and Hay 1996b). Other factors, such as ultraviolet radiation and the availability of nutrients and light, also affect the production of these compounds (Cronin and Hay 1996a; Cronin and Lodge 2003). We cut the *M. spicatum* stems to fit the aquaria, which probably accelerated the release of chemicals. However, autofragmentation of *M. spicatum* plants is typical after flowering periods, as is damage by waves and human activities such as mechanical weed harvesting and boat traffic (Nichols and Shaw 1986). Thus, cut *M. spicatum* stems do commonly occur in the nature. Further, aquatic angiosperms are considered leaky since they have a reduced cuticula, no stomata, and relatively loose cell connections, which facilitate the release of organic compounds (reviewed in Gross 2003) even from intact plants. Still, the toxicity and repellence of *M. spicatum* to mysids and sticklebacks seems surprising because none of these species feed on aquatic macrophytes (Mauchline 1980; Hangelin and Vuorinen 1988), and they should, therefore, not be targets of antiherbivore substances. On the other hand, mysids are very sensitive to chemical contaminants such as trace metals and pesticides (Nimmo and Hamaker 1982; Roast et al. 1998) and may therefore be sensitive to natural toxins as well. Sublethal contaminant concentrations suppress swimming and feeding activity of mysids (Nimmo and Hamaker 1982) and interrupt swarming (Roast et al. 1998), which is an efficient predator avoidance strategy (Flynn and Ritz 1999). Notably, in the lower vegetation to water ratio of our behavior experiments the toxin concentration was too low to induce mortality but high enough to induce behavioral responses in *N. integer* as well as stickleback larvae. The biomass of *M. spicatum* used in the mortality experiments fits well within values of maximum site biomasses reported from North American lakes (Nichols and Shaw 1986), as does the stem density to maximum field densities in the study area (pers. obs.).

M. spicatum proved to be an unsuitable refuge for littoral mysids and three-spined stickleback larvae. *M. spicatum*'s unsuitability as a habitat has also been observed in North America, where the abundances of invertebrates are lower on *M. spicatum* vegetation and in the benthos beneath a *M. spicatum* community than on and beneath other macrophytes (Nichols and Shaw 1986). In our study, the stickleback larvae did use it as a refuge from predation to some extent, but significantly less than artificial vegetation. Also *N. integer* avoided *M. spicatum* and spent less time within it than within artificial vegetation. However, predator signals did not induce an increase in the refuge use of *N. integer*, and thus it cannot be considered as effective hiding. Only the behavior of *P. flexuosus* was not affected by *M. spicatum*. This may be explained by the predator avoidance strategy of *P. flexuosus*. It lives in close association with macrophytes throughout its life cycle and is highly dependent on them as a predation refuge because, unlike *N. integer* or three-spined

sticklebacks, it does not avoid predation by decreased activity (Lindén et al. 2003) or swarming (Mauchline 1971; Fitzgerald and Wootton 1993). We therefore suggest that when facing a trade-off situation between avoiding predation and avoiding *M. spicatum*, *P. flexuosus* chooses to hide in the vegetation despite its toxicity. The group behavior of *N. integer* and stickleback larvae may somewhat differ from solitary behavior, but the repellent effect of *M. spicatum* is not likely to change.

In addition to the effects of *M. spicatum* on the habitat choice of stickleback larvae, the presence of *M. spicatum* suppressed their swimming and feeding activities. An especially dramatic effect was found in the attack rate, which declined to a very low level even in the absence of predator signals. Larval fish need to grow fast to be able to survive the following winter and to outgrow predators (reviewed in Pedersen 1997); thus, sufficient feeding is of utmost importance. The complex morphology of *M. spicatum* may lower the feeding efficiency of fish (Coull and Wells 1983), but the high mortality of mysids in the presence of *M. spicatum* and the fact that the feeding of stickleback larvae was unaffected by similarly structured *C. tomentosa* (Lehtiniemi in press) suggest a strong chemical interaction. Furthermore, zooplankton prey may have been avoiding the *M. spicatum* vegetation due to the excreted chemicals (Pennak 1973; Lauridsen and Lodge 1996), further increasing the motivation of larvae to stay in open water area.

Concurrent with earlier studies (e.g., Bishop and Brown 1992), our results show that three-spined stickleback larvae are able to detect predation risk and adjust their behavior accordingly. The response of sticklebacks to predator signals differs from the one of *N. integer* and *P. flexuosus*. With mysids, neither chemical nor visual predator signals alone were sufficient to produce a behavioral response (Lindén et al. 2003). In the present study, chemical signals alone could produce a significant response in swimming and feeding activity of stickleback larvae, though not in their habitat choice. This is hardly surprising, considering the more sophisticated sensory systems of vertebrates.

Our results indicate that *M. sibiricum* and *C. tomentosa* provide suitable refuges for littoral planktivores such as mysids and three-spined stickleback larvae, whereas *M. spicatum* is most harmful. Further studies on the lethality and repellence of *M. spicatum* to planktivores in the field need to be conducted. Notably, *Chara* spp. are very sensitive to anthropogenic habitat changes, such as eutrophication, and they are rapidly declining in many areas (Schubert and Yousef 2001), including the archipelago of southwest Finland (Munsterhjelm 1997; Koistinen and Munsterhjelm 2001). In contrast, *M. spicatum* thrives in eutrophied conditions and it is presently spreading to new areas (Nichols and Shaw 1986; Munsterhjelm 1997). The sublethal effects of *M. spicatum* on the behavior of mysids and three-spined stickleback larvae, which cause them to spend more time outside the refuge, exposed to predators, and to feed less efficiently, may prove to be detrimental in the long run. The availability of high-quality nursery habitats to larval fish is crucial to the recruitment and production of fish populations (Urho 2002). We conclude that the anthropogenic changes in macrophyte species composition that favor *M. spicatum* may cause major

changes in populations of three-spined sticklebacks and mysids. Through the effects on the feeding and distribution of their fish predators, the whole structure of the littoral ecosystems of the Baltic Sea may be affected.

References

- AGAMI, M., AND Y. WAISEL. 1985. Inter-relationships between *Najas marina* L. and three other species of aquatic macrophytes. *Hydrobiologia* **126**: 169–173.
- ANTHONI, U., C. CHRISTOPHERSEN, J. ØGÅRD MADSEN, S. WIUM-ANDERSEN, AND N. JACOBSEN. 1980. Biologically active sulphur compounds from the green alga *Chara globularis*. *Phytochemistry* **19**: 1228–1229.
- BANKOVA, V., AND OTHERS. 2001. Secondary metabolites and lipids in *Chara globularis* Thuill. *Hydrobiologia* **457**: 199–203.
- BERGER, J., AND M. SCHAGERL. 2003. Allelopathic activity of *Chara aspera*. *Hydrobiologia* **501**: 109–115.
- BISHOP, T. D., AND J. A. BROWN. 1992. Threat-sensitive foraging by larval threespine sticklebacks (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.* **31**: 133–138.
- BURKS, R. L., E. JEPPESEN, AND D. M. LODGE. 2000. Macrophyte and fish chemicals suppress *Daphnia* growth and alter life-history traits. *Oikos* **88**: 139–147.
- , ———, AND ———. 2001. Littoral zone structures as *Daphnia* refugia against fish predators. *Limnol. Oceanogr.* **46**: 230–237.
- COULL, B. C., AND J. B. J. WELLS. 1983. Refuges from fish predation: Experiments with phytal meiofauna from the New Zealand rocky intertidal. *Ecology* **64**: 1599–1609.
- CRONIN, G., AND M. E. HAY. 1996a. Susceptibility to herbivores depends on recent history of both the plant and animal. *Ecology* **77**: 1531–1543.
- , AND ———. 1996b. Induction of seaweed chemical defenses by amphipod grazing. *Ecology* **77**: 2287–2301.
- , AND D. M. LODGE. 2003. Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia* **137**: 32–41.
- DHILLON, M. S., M. S. MULLA, AND Y. HWANG. 1982. Allelochemicals produced by the hydrophyte *Myriophyllum spicatum* affecting mosquitoes and midges. *J. Chem. Ecol.* **8**: 517–526.
- EJDUNG, G. 1998. Behavioural responses to chemical cues of predation risk in a three-trophic-level Baltic Sea food chain. *Mar. Ecol. Prog. Ser.* **165**: 137–144.
- FITZGERALD, G. J., AND R. J. WOOTTON. 1993. The behavioural ecology of sticklebacks, p. 169–196. *In* T. J. Pitcher [ed.], *Behaviour of teleost fishes*. Chapman & Hall.
- FLYNN, A. J., AND D. A. RITZ. 1999. Effect of habitat complexity and predatory style on the capture success of fish feeding on aggregated prey. *J. Mar. Biol. Assoc. UK* **79**: 487–494.
- GROSS, E. M. 2003. Allelopathy in aquatic autotrophs. *Crit. Rev. Plant Sci.* **22**: 313–339.
- HANGELIN, C., AND I. VUORINEN. 1988. Food selection in juvenile three-spined sticklebacks studied in relation to size, abundance and biomass of prey. *Hydrobiologia* **157**: 169–177.
- HASLER, A. D., AND E. JONES. 1949. Demonstration of the antagonistic action of large aquatic plants on algae and rotifers. *Ecology* **30**: 359–364.
- JACOBSEN, N., AND L. PEDERSEN. 1983. Synthesis and insecticidal properties of derivatives of propane-1,3-dithiolane (analogues of the insecticidal derivatives of dithiolane and triethane from the alga *Chara globularis* Thuillier). *Pestic. Sci.* **14**: 90–97.
- JAKOBSEN, P. J., G. H. JOHNSEN, AND P. LARSSON. 1988. Effects of predation risk and parasitism on the feeding ecology, habitat

- use, and abundance of lacustrine threespine stickleback (*Gasterosteus aculeatus*). *Can. J. Fish. Aquat. Sci.* **45**: 426–431.
- JOHNSON, G. D., AND M. S. MULLA. 1983. An aquatic macrophyte affected nuisance chironomid midges in a warm-water lake. *Environ. Entomol.* **12**: 266–269.
- KOISTINEN, M., AND R. MUNSTERHJELM. 2001. Charophytes of the Finnish coastal waters. *Schr.reihe Landschaftspflege Naturschutz* **72**: 27–29.
- LAURIDSEN, T. L., AND D. M. LODGE. 1996. Avoidance by *Daphnia magna* of fish and macrophytes: Chemical cues and predator-mediated use of macrophyte habitat. *Limnol. Oceanogr.* **41**: 794–798.
- LEHTINIEMI, M. In press. Swim or hide—predator cues cause species specific reactions in young fish larvae. *J. Fish. Biol.*
- LEVIN, D. A. 1971. Plant phenolics: An ecological perspective. *Am. Nat.* **105**: 157–181.
- LIMA, S. L., AND L. M. DILL. 1990. Behavioral decisions made under the risk of predation: A review and prospectus. *Can. J. Zool.* **68**: 619–640.
- LINDÉN, E., M. LEHTINIEMI, AND M. VIITASALO. 2003. Predator avoidance behaviour of Baltic littoral mysids *Neomysis integer* and *Praunus flexuosus*. *Mar. Biol.* **143**: 845–850.
- MATHESON, R. 1930. The utilization of aquatic plants as aids in mosquito control. *Am. Nat.* **64**: 56–86.
- MAUCLINE, J. 1971. Seasonal occurrence of mysids (Crustacea) and evidence of social behaviour. *J. Mar. Biol. Assoc. UK* **51**: 809–825.
- . 1980. The biology of mysids and euphasiids. *Adv. Mar. Biol.* **18**: 1–369.
- MILINSKI, M., AND R. HELLER. 1978. Influence of a predator on the optimal foraging behaviour of sticklebacks (*Gasterosteus aculeatus* L.). *Nature* **275**: 642–644.
- MOSS, B., R. KORNIJOW, AND G. J. MEASEY. 1998. The effects of nymphaeid (*Nuphar lutea*) density and predation by perch (*Perca fluviatilis*) on the zooplankton communities in a shallow lake. *Freshw. Biol.* **39**: 689–697.
- MUNSTERHJELM, R. 1997. The aquatic macrophyte vegetation of flads and gloes, S coast of Finland. *Acta Bot. Fenn.* **157**: 1–68.
- NAKAI, S., Y. INOUE, AND M. HOSOMI. 2001. Algal growth inhibition effects and inducement modes by plant-producing phenols. *Water Res.* **35**: 1855–1859.
- NICHOLS, S. A., AND B. H. SHAW. 1986. Ecological life histories of the three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus* and *Elodea canadensis*. *Hydrobiologia* **131**: 3–21.
- NIMMO, D. R., AND T. L. HAMAKER. 1982. Mysids in toxicity testing—a review. *Hydrobiologia* **93**: 171–178.
- PEDERSEN, B. H. 1997. The cost of growth in young fish larvae, a review of new hypotheses. *Aquaculture* **155**: 259–269.
- PENNAK, R. W. 1973. Some evidence for aquatic macrophytes as repellents for a limnetic species of *Daphnia*. *Int. Rev. Gesamten Hydrobiol.* **58**: 569–576.
- REIMCHEN, T. E. 1994. Predation and morphological evolution in threespine stickleback, p. 240–276. In M. A. Bell and S. A. Foster [eds.], *The evolutionary biology of the threespine stickleback*. Oxford Univ. Press.
- ROAST, S. D., R. S. THOMPSON, J. WIDDOWS, AND M. B. JONES. 1998. Mysids and environmental monitoring: A case for their use in estuaries. *Mar. Freshw. Res.* **40**: 827–832.
- ROMARE, P., AND L.-A. HANSSON. 2003. A behavioral cascade: Top-predator induced behavioral shifts in planktivorous fish and zooplankton. *Limnol. Oceanogr.* **48**: 1956–1964.
- SCHUBERT, H., AND M. A. M. YOUSEF. 2001. Charophytes in the Baltic Sea—threats and conservation. *Schr.reihe Landschaftspflege Naturschutz* **72**: 7–8.
- SINGLETON, V. L., AND F. H. KRATZER. 1969. Toxicity and related physiological activity of phenolic substances of plant origin. *J. Agric. Food Chem.* **17**: 497–512.
- SPENCER, D. F., AND G. G. KSANDER. 1999. Phenolic acids and nutrient content for aquatic macrophytes from Fall River, California. *J. Freshw. Ecol.* **14**: 197–209.
- SUNDELL, J. 1994. Dynamics and composition of littoral fish fauna along the coast of SW-Finland. *Aqua Fenn.* **24**: 37–49.
- THIEL, R. 1996. The impact of fish predation on the zooplankton community in a southern Baltic bay. *Limnologica* **26**: 123–137.
- URHO, L. 2002. The importance of larvae and nursery areas for fish production. Ph.D. thesis, Univ. of Helsinki.
- VIITASALO, M., T. KIØRBOE, J. FLINKMAN, L. W. PEDERSEN, AND A. W. VISSER. 1998. Predation vulnerability of planktonic copepods: Consequences of predator foraging strategies and prey sensory abilities. *Mar. Ecol. Prog. Ser.* **175**: 129–142.
- , I. VUORINEN, AND S. SAESMAA. 1995. Mesozooplankton dynamics in the northern Baltic Sea: Implications of variations in hydrography and climate. *J. Plankton Res.* **17**: 1857–1878.

Received: 28 March 2004

Accepted: 9 November 2004

Amended: 19 November 2004