



The macrophyte, *Stratiotes aloides*, protects larvae of dragonfly *Aeshna viridis* against fish predation

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Abstract

Predation could be one force determining which contemporary species occupy a certain habitat. *Aeshna viridis* is an endangered dragonfly species with a larval distribution strongly associated with lakes where the water plant, water soldier, *Stratiotes aloides*, occurs. In this study, the larvae were almost exclusively found in patches of *S. aloides*. To study larval association with *S. aloides* further, we conducted a series of laboratory experiments. Behavioural experiments indicated that larvae preferred *S. aloides*. *Aeshna viridis* larvae were nocturnal and rather inactive. Larvae on *S. aloides* were less susceptible to predation by the perch, *Perca fluviatilis*, than larvae on another water plant, *Myriophyllum alterniflorum*. According to our study it seems that occurrence of *S. aloides* may limit the distribution of *A. viridis*, and the protection of refuges may be essential in planning the conservation of this endangered species.

Introduction

It has been suggested recently that predation could increase local extinction probabilities and decrease the prospects of local colonization of species (Schoener and Spiller 1995; Crooks and Soulé 1999). Predators may drive some prey types into local extinction, and alter the relative and absolute abundances and species diversity of prey (Sih 1987; Schoener and Spiller 1995). Odonate larvae are frequently preyed upon by fish (e.g., Crowder and Cooper 1982; Martin et al 1991; Diehl 1993). Many researchers have suggested that predation by fish and other dragonflies is a strong force determining which odonate species can occupy a certain habitat (reviews in Johnson 1991; McPeck 1998; Corbet 1999). In several experimental studies with dragonflies it has been noticed that the

presence of macrophytes can reduce the risk of predation (see review in Corbet 1999). For example, larvae of *Pachydiplax longipennis* (Burmeister) used regions of the rooted macrophyte *Sagittaria platyphylla* (Engelm.) in a non random fashion, preferring the leaf axils where they were less exposed to predation by fishes (Wellborn and Robinson 1987). When deprived of food, larvae moved to the upper parts of the foliage but rested head downwards, jetting rapidly to the axil when disturbed. However, the distribution of the larvae among other plants suggested that there was no active preference for *S. platyphylla* as a substrate (Wellborn and Robinson 1987).

Aeshna viridis (Eversman) is an endangered species in Europe (Valtonen 1997) and is protected by the nature conservation legislation of the European Union. *A. viridis* is a dragonfly species whose larval

distribution in northern Europe is strongly associated with a water macrophyte species, *Stratiotes aloides* (Askew 1988). *Stratiotes aloides* typically exists in very shallow parts of the littoral zone as an emerged form, as well as in deeper parts as a submerged form. Moreover, there is regular 'migration' of the plants between the bottom and the water surface during the vegetation cycle (e.g., Higler 1977). During the last few decades, *S. aloides* has shown a general decline in distribution. Smolders et al. (1996) suggested that a possible cause for the decline might be multiple environmental stresses, including light competition by non-rooting species, iron limitation and sulphide and ammonium toxicity, which result from modern agricultural practices. In more urban areas *S. aloides* has been removed by anglers because it is liable to take over a pond with its dominant growth type (Gibbons 1986). With the decline of *S. aloides* in Europe (e.g., Roelofs 1991; Smolders et al. 1996; de Jong 2000; de Jong and Verbeek 2001), *A. viridis* has also become scarcer and has disappeared from many former distribution areas (e.g., Gibbons 1986). Certain conservation actions are taking place to improve the quality of existing suitable water bodies and to develop new biotopes, both for *S. aloides* and *A. viridis* (de Jong 2000; de Jong and Verbeek 2001).

Female *A. viridis* mainly lay eggs on the leaves of *S. aloides* (L.) and occasionally on some other water plants, e.g., *Typha* spp. and *Sparganium* spp. (Rantalainen and Kanervo 1928; Askew 1988). Adults of *A. viridis* have been found occasionally by water without *S. aloides* (e.g., Rantalainen and Kanervo 1928), but the larvae have only been recorded in lakes and ponds with this macrophyte (e.g., Valle 1952; Norling and Sahlen 1997). This is possibly because the spiny leaves of *S. aloides* protect the larvae against fish predation. In previous studies it has been noticed that herbivorous fish avoid the *S. aloides* as a food in spite of its dominance in the littoral (e.g., Prejs 1973), and that *S. aloides* protection is stronger against fish than predatory invertebrates (Prejs et al. 1997).

In this study, we set out to examine whether the association of the dragonfly, *A. viridis*, with water soldier, *S. aloides*, really exists, and whether this association is related to protection against fish predation. We addressed four specific issues in this study. First, we studied whether *A. viridis* larvae and *S. aloides* co-occurred in eutrophic lakes in Finland. Although *A. viridis* is widespread and it occurs mainly in waters with *S. aloides* (Valle 1952; Valtonen 1997), empirical evidence of the relationship between the

two species is still limited. Second, we conducted a laboratory experiment to test whether larvae preferred *S. aloides* over another water plant, *Myriophyllum alterniflorum* (DC.), which was abundant in lakes containing *A. viridis* larvae. Third, we examined the circadian activity pattern of *A. viridis* larvae. Fourth, we conducted aquarium experiments to examine how fish predation on *A. viridis* larvae was influenced by the presence of *S. aloides* and *M. alterniflorum*. *S. aloides* has spine-toothed leaves that form large rosettes (Prejs 1973; Hämet-Ahti et al. 1984) and may give better protection against fish predation than *M. alterniflorum*. *Myriophyllum* plant/clump provides fairly good visual refuges but is a less effective physical shelter against fish predation than *S. aloides*.

Methods

Field study

To determine the degree to which *A. viridis* and *S. aloides* co-occurred, we sampled 15 eutrophic lakes in southern Finland during the end of June and early July, 2000. Eight of 15 lakes supported *S. aloides*. From each lake we took 24 sweep samples with a standard pond net (mesh size 0.4 mm), each sweep lasting 2 minutes. The sample stations were classified into three habitat categories: (i) upper littoral zone 0–0.5 m, (ii) lower littoral zone 0.5–1.0 m, and (iii) *S. aloides* patches. If the lake did not support *S. aloides*, habitat (iii) was lower littoral zone vegetation, where *S. aloides* typically exists. We took eight samples from each zone, including both the vegetation and the bottom sediment, and we pooled the data from each set of eight samples for statistical analysis.

For the behaviour and predation experiments, we collected 400 *A. viridis* larvae, 15 to 20 mm in body length, from Lake Saarioisjärvi (61°10' N; 25°03' W) in southern Finland in June and August, 1999. Lake Saarioisjärvi is a small, shallow eutrophic lake supporting dense beds of *S. aloides* and a large population of *A. viridis*. During the same day, ca. 100 *S. aloides* plants were selected randomly and removed from a large patch of vegetation. Each plant was examined and all odonate larvae were collected until a sufficient number of larvae had been obtained. After running the experiments, all surviving *A. viridis* larvae were released back to the Lake Saarioisjärvi.

Laboratory experiments (see below) were performed at Konnevesi Research Station in Central

Finland (62°37' N, 26°20' E). When not being used in trials, the larvae were kept in a large plastic 60 l bowl containing *S. aloides* rosettes and fed with *Asellus aquaticus* L. and *Baetis* sp. larvae daily. During the trials larvae were not fed and no food was available in the aquarium.

Behavioural experiments

Our behaviour experiments tested whether *A. viridis* larvae consistently prefer *S. aloides*. We also examined the circadian activity pattern of the larvae. For the experiments, we used thirteen 60 l glass aquaria filled with water from the meso-oligotrophic Lake Konnevesi. Each aquarium was used twice to give a total of 26 replicates. Each aquarium contained two water plants. A *S. aloides* rosette was placed in one corner, and a clump of *M. alterniflorum* in the opposite corner. The size of the plant, and thus the relative macrophyte cover, on each side of an aquarium was equal to reduce differences in spatial heterogeneity. The bottom of the aquarium was covered with fine sand. Only one larva was used in each aquarium, and each larva was used only once. The larvae were placed in a random position on the surface of the water of each aquarium and were left to acclimatize for 24 h before the start of a trial. During the trial each aquarium was surveyed for three 1-minute periods (09:00, 16:00 and 23:00 h) to record the position and activity of the larvae. The larvae were recorded as active if they were moving (e.g., crawling, swimming, scratching) during the surveillance. The experimental room was illuminated between 4.00 to 22.00. During night trials, observation was facilitated by a red dark-room light, which was on only during the surveillance periods.

Predation experiment

To assess the value of *S. aloides* as a refuge, we conducted a predation experiment using perch, *Perca fluviatilis* (L.), as a predator. Perch is the most common and widespread fish species in Finland and is an important predator of many aquatic insects (e.g., Rask 1986; Guti 1993). Perch is also common in Lake Saarioisjärvi, from where we obtained the dragonfly larvae. In the experiment, we used four treatments: (1) *M. alterniflorum* and no fish (control for mortality and cannibalism), (2) *S. aloides* without fish (control for mortality and cannibalism), (3) *M. alterniflorum* with fish and (4) *S. aloides* with fish. Experiments

were performed in large, round plastic fish-holding tanks (diameter 138 cm, 120 cm high, water level 100 cm). Either five clumps of *M. alterniflorum* or five *S. aloides* rosettes were placed in each tank. Total macrophyte cover in each treatment was equal to remove differences in spatial heterogeneity (see Crowder and Cooper 1982). Water was pumped into the tanks from Lake Konnevesi. Water temperature during the experiments was about 12 °C and the experimental room was illuminated by light between 04:00 to 22:00 h. Fishes were captured from Lake Keitele and Lake Konnevesi and maintained in separate holding tanks for several days prior to experiments. Eight of the tanks were used in the experiment at one time, and one was used for acclimating the fishes before the experiment. Treatments were randomly assigned to the eight experimental tanks.

Ten *A. viridis* larvae (instar F-2) were used in each treatment. This density was similar to that observed in *S. aloides* patches in Lake Saarioisjärvi (M. Rantala, *personal observation*). The larvae were allowed to acclimatize for 15 h before introducing the predators. Five perch, length ranging from 15 to 20 cm, were placed in each tank. Fishes were selected randomly from a group of 20 to 30 kept in a separate holding tank, and each was used only once. We started the experiments at 09:00 h and ran them for 24 hours. At the end of each trial, the fishes were removed, the tanks were emptied, and their entire contents were sorted carefully to discover all surviving larvae. We carried out ten trials in June 1999 and three trials in August 1999, for a total of 13 replicates of each treatment combination. After the trials, the perch and the larvae were returned to their home lakes. In statistical procedures, we used the Bonferroni correction to reduce the risk of achieving significant results by chance alone (see Lessels and Boag 1987).

Results

Field study

Larvae of *A. viridis* were strongly associated with lakes containing *S. aloides*. Five out of eight lakes supporting *S. aloides* were also occupied by *A. viridis*, and we found no *A. viridis* larvae in the seven lakes without *S. aloides* (Fisher test, $P = 0.0256$). Within the eight lakes containing *S. aloides*, larvae were located almost exclusively in *S. aloides* patches

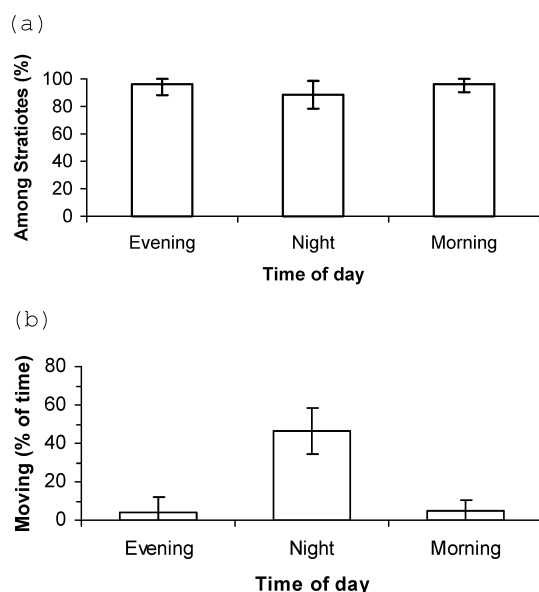


Figure 1. (a) Percentage of observations (average \pm 95% confidence interval) of *A. viridis* larvae on *S. aloides* compared with *M. alterniflorum*, and (b) the percentage of larvae active at different times of day. For each treatment, $n = 26$.

(Friedman's ANOVA, $\chi^2 = 9.50$, $df = 2$, $p = 0.009$, a lake as a block). Only one larva out of 43 was found outside a *S. aloides* patch. We also noticed that *A. viridis* larvae tended to be located in the leaf axil region.

Behavioural experiments

In general, *A. viridis* larvae showed a strong preference for *S. aloides* over *M. alterniflorum* (Figure 1a). Larvae were found much more frequently in patches of *S. aloides* than in *M. alterniflorum* at all times of day (Wilcoxon test, evening, $z = -4.71$, $p < 0.001$, night, $z = -4.25$, $p < 0.001$, morning, $z = -4.90$, $p < 0.001$, Figure 1a). There were no differences in habitat preference between times of day (Friedman's ANOVA, $\chi^2 = 4.68$, $df = 2$, $p = 0.097$, Figure 1a). *A. viridis* larvae were more active during the night than in the morning or evening (Friedman's ANOVA $\chi^2 = 31.65$, $df = 2$, $p < 0.001$, Figure 1b). During the day the larvae tended to locate in the leaf axil regions, protected by the spine-toothed leaves. Swimming, scratching and walking were performed mainly at night.

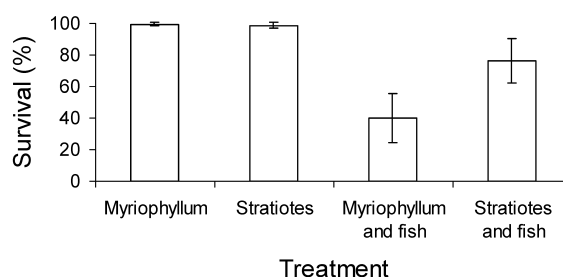


Figure 2. The average (\pm 95% confidence interval) percentage of survived *A. viridis* larvae in each treatment. For each treatment, $n = 13$.

Predation experiment

Survival of the larvae differed among treatments (Kruskal-Wallis ANOVA, $H = 37.24$, $df = 3$, $p < 0.001$, Figure 2). Survival of *A. viridis* larvae was lower in fish treatments than in treatments without fish (Mann-Whitney *U*-test, $z = -5.63$, $n_1 = n_2 = 26$, $p < 0.001$, Bonferroni corrected). Almost all larvae survived in non-predatory treatments (mean = 9.88, $SD = 0.33$, $n = 26$), but only slightly more than half of the larvae survived in fish treatments (mean = 5.81, $SD = 3.03$, $n = 26$). Deaths of larvae in treatments without fish appeared to be mainly the result of cannibalism. In treatments containing fishes, survival of larvae was higher in tanks with *S. aloides* than in tanks with *M. alterniflorum* ($U = 24$, $n_1 = n_2 = 13$, $p = 0.008$, Bonferroni corrected, Figure 2), indicating that the presence of *S. aloides* reduced the risk of fish predation.

Discussion

Our study supported the idea that *A. viridis* larvae tend to be located in patches of *S. aloides* in the field, and thus is consistent with earlier records (e.g., Valle 1952; Norling 1971). To our knowledge, *A. viridis* is the only dragonfly species demonstrated to be so tightly associated with a certain plant species. We also showed in the behavioural experiments that larvae actively prefer *S. aloides* over *M. alterniflorum*. Although there could be many explanations for the larval preference of *S. aloides*, two seem most plausible. First, the distribution of *A. viridis* may simply reflect non-random distribution of prey. If so, larvae might prefer *S. aloides* because it tends to provide sites of high density of suitable food items (Higler 1977;

Prejs et al. 1997). Such a preference could not be an active response to food density, however, because no food items were available to larvae during our behavioural experiments. Second, *S. aloides* patches may be sites of low predation risk. If so, larvae might select them because of their relatively high refuge value. This would be an active response to predation risk (Hopper 2001) or fixed (canalised) behaviour (Sih 1987). It may be that larvae of *A. viridis* can detect a threat of predation via chemical cues of fish from water. In a previous study larvae of *Pachydiplax longipennis* have been found to be sensitive to waterborne cues of fish in their environment (Hopper 2001). There may be other benefits to larvae from selecting patches of *S. aloides*, but from our predation experiment it is likely that patches of *S. aloides* are at least sites of relatively low predation risk. On the other hand the larval preference of *A. viridis* for *S. aloides* may be a fixed (canalised) behaviour and does not require the presence of a fish or chemical cues as a triggering mechanism. Fixed antipredator behaviours are expected to evolve when the risk of predation is predictably high (Sih 1987). For example, ambush foraging and low use of exposed sites are fixed traits of many other odonates that coexist with fish (see Johnson 1991). In addition, it has been found that larvae of *Pachydiplax longipennis*, which are especially vulnerable to fish predation (Morin 1984), also occupy refuges among aquatic plants in absence of fish (Wellborn and Robinson 1987, see also Hopper 2001). Our experimental data suggest that fish predation can cause habitat selection of *A. viridis* larvae, but we emphasize that this does not exclude the possibility that egg deposition by the adult *A. viridis* female to *S. aloides* is not important. Possibly the young dragonfly larvae are strongly associated with their hatching plant species and for that reason always selected *S. aloides* plant in our experiment.

Our behaviour experiment showed that larvae of *A. viridis* are nocturnal. Nocturnal behaviour is common among odonates coexisting with fish (Johnson 1991). Most fish species are visual predators that react to movements of prey organisms (Persson 1983). Thus, nocturnal activity of *A. viridis* might be interpreted as an adaptation to avoid visual predators. In addition, larvae of *A. viridis* are green (e.g., Norling and Sahlen 1997) and closely resemble their immediate background. Because of their cryptic coloration they are well camouflaged from fish predators. Even when active, *A. viridis* larvae move very little. Reduced activity *per se* is a typical antipredatory response that

presumably reduces the frequency of encounter with a predator and the likelihood of being detected and recognized as prey (Sih 1987). Although these adaptations may reduce the susceptibility to fish predation, they may make *A. viridis* more vulnerable to predation by other predators, such as other large dragonflies that dominate many fish-free sites (see Johnson 1991).

The data presented here indicate that patches of *S. aloides* serve as refuges from predation by fish. Our results also indicated that the survival of larvae was much better in tanks with *S. aloides* than in tanks with *M. alterniflorum*. This is not surprising since the young leaves of *S. aloides* are so spiny that even fish tend to avoid them. A strong preference for leaf axil regions may further decrease the vulnerability of *A. viridis* larvae to fish predation (see Wellborn and Robinson 1987).

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