FORAGING BEHAVIOUR OF THE DAMSELFLY LARVA
PYRRHOSOMA NYMPHULA (SULZER)
IN RESPONSE TO PREDATOR PRESENCE
(ZYGOPTERA: COENAGRIONIDAE)

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The trade off between foraging and predator avoidance was studied. In the presence of a larva of the predatory *Aeshna juncea*, *P. nymphula* was found to reduce foraging activity significantly. *P. nymphula* reduced foraging activity in response to chemical stimuli from *A. juncea* but not in response to visual stimuli. Foraging activity was further reduced when the diet of *A. juncea* was changed from chironomid larvae to *P. nymphula*. This suggests that predators are detected chemically and are chemically labelled by their diet. Foraging activity was found to increase with starvation level after 48 h without access to food, with a further increase after 72 h of starvation. The presence of chemical stimuli from conspecific fed predators delayed the increase in foraging activity until 72 h of starvation. These results have implications for larval survival and adult reproductive fitness.

INTRODUCTION

Optimal foraging theory suggests that the goal of a foraging organism is to maximise the net intake of energy in order to enhance fitness (MacARTHUR & PIANKA, 1966). Many studies have shown that the presence of a predator alters foraging behaviour (e.g. SIH, 1980; HEADS, 1985, 1986), maximising feeding rate and minimising predation (LIMA & DILL, 1990).

Anti-predator mechanisms may be fixed or reactive, both of which are important in reducing predation risk in many prey species (PIERCE, 1988). An example of a reactive response is reduced foraging activity, which may be categorised into moving or refuging. Decreasing activity following a heightened predation risk is well-established across diverse taxa (LIMA & DILL, 1990; LIMA, 1998). Many species

are known to decrease foraging, increase use of refuges or indeed a combination of both. In damselfly larvae, the duration and type of activity adopted has been shown to influence their vulnerability to predation (HEADS, 1985).

Foraging activity may both alert a predator to a prey individual's whereabouts and increase the chance of the prey encountering a potential predator (LIMA, 1998). Therefore, reducing movement should be an important antipredator behaviour adopted by many species. In order to reduce activity a damselfly larva may alter its foraging strategy. For example it may adopt a 'sit and wait' strategy rather than an 'active' foraging mode, depending on its prey type and prey abundance (JOHANS-SON, 1991). However, adopting a 'sit and wait' strategy often or over a long period may result in a slower growth rate overall (HARVEY & WHITE, 1990).

A reduced growth rate may mean a life history change, with a lower foraging rate and slower growth ultimately leading to delayed emergence and lower survivorship of larvae (HEADS, 1985; 1986). Predation has been shown to prolong maturation of damselfly larvae by 18%, and may result in larvae having to overwinter twice, instead of just once (HEADS, 1986). Recent studies have shown that the relationship between reduced foraging efficiency and mortality risk is essential for understanding the size and time of maturation of larvae (STOKS & JOHANSSON, 2000). Individuals may suffer fitness losses due to submaximal feeding rates as larvae, resulting in a smaller size at moult, lower fecundity and reduced reproductive fitness in adults (HEADS, 1986), which may in turn lead to decreased reproductive success (JOHANSSON, 1993b). Similar results have been found in other invertebrates. In mayflies, for example, a smaller size at maturation may reflect predator-induced stress encountered during the larval stage (LIMA, 1998). ABRAMS & ROWE (1996) suggested that flexible life cycle species should decrease foraging in the presence of predators and (STOKS & JOHANSSON, 2000) that fixed one year life cycle species would be expected to increase foraging activity.

Larvae may also change microhabitat in response to predation. Seeking refuges or moving microhabitats is a fixed behaviour. In response to predator presence the larvae of the damselfly, *Ischnura elegans*, have been shown to move to a less profitable feeding patch within a habitat (HEADS, 1986; JOHANSSON, 1991). Additionally, *I. elegans* were shown to change perch sites less often and move shorter distances in the presence of a predatory water boatman, *Notonecta glauda* (LIMA & DILL, 1990), demonstrating that predators may greatly curb activity and foraging efficiency. Movement of damselfly larvae, *Ischnura verticalus*, to dark substrate areas is also enhanced by predation threat (LIMA, 1998), demonstrating that predation risk may affect space usage by prey individuals, thereby influencing the nature of ecological systems.

LIMA (1998) stated that an assessment of predation risk must in some way form the basis for antipredator decision-making. Behavioural responses of invertebrates may depend on the perceptive abilities of the predator and prey (CULP et al., 1991), as 'decisions' may depend on the ability to detect predatory attacks. The predator

may be detected visually, mechanically or chemically or in a combination of these ways.

Odonata are known to have mechanoreceptors and in 'sprawler' odonates such as *Cordulia* mechanoreception forms the principal means of prey detection (CORBET, 1999). Mechanoreceptors may be housed on the antennae and on the legs and used to detect water vibrations (CORBET, 1999). Indeed RICHARDS & BULL (1990) found that larvae of *Hemicordulia tau* capture tadpoles in complete darkness using no visual cues. It is plausible to suggest that mechanoreception also plays an active role in predator detection. Other insect larvae such as mosquito larvae (KOPER-SKI, 1997) and mayfly larvae have been shown to use mechanoreceptors to detect turbulence created by potential predators (LIMA & DILL, 1990).

Predation is an important factor influencing foraging behaviour. The objective of this study was to determine the way in which larvae of the damselfly *P. nym-phula* assess predation risk, presented with different predator stimuli, and the effect this has on their foraging activity. Dragonfly larvae were the chosen predator as in fishless ponds they are often the top predators in the food web (JOHNSEN, 1991; McPEEK, 1990; JOHANSSON, 1993a). It has been shown in many studies that aeshnid predation may have a major impact on other odonate larvae (JO-HANSSON, 1993a). Studies on *Enallagma boreale*, damselfly larvae have shown that up to 75% of natural larval mortality can be directly attributed to dragonfly predation (McPEEK, 1997).

The effect of starvation level was also considered, with respect to the willingness of larvae to expose themselves to predation risk by foraging.

SPECIES

Pyrrhosoma nymphula larvae are dark, stout and robust. They rest on mud bottoms and amongst vegetation and are found in ponds, lakes, ditches and sometimes even brackish water (BROOKS, 2002). Although the only species of the genus found in Europe, *P. nymphula* is very common almost everywhere and can occur in very large numbers (D'AGUILAR et al., 1986). The larvae are usually semivoltine, completing a generation in two years, but, in areas of high competition, predation or food shortage, larvae can lower activity and take an extra season to develop (HARVEY & WHITE, 1990). BENNETT & MILL (1993) discuss the possible conditions necessary for univoltinism.

Larvae of *Aeshna juncea* are large and voracious predators. They are found in a wide variety of still waters from moorland bog pools to large lakes. Young larvae are found near the water surface clinging to plants; larger larvae usually occur on the bottom of pools clinging to low stems. The larval stage extends over at least three seasons, probably longer in colder environments (BROOKS, 2002).

METHODS

Both experimental species were collected from Limekilns pond, Palaceriggs Country Park, near Cumbernauld (NS 786736) in late October 2002. All larvae were identified using a dichotomous identification key (BROOKS, 2002). Eye capsule width of each *P. nymphula* individual was measured and the larvae assigned to instars. Only final instar larvae were used in experiments.

All animals and experiments were maintained in a controlled temperature room at 8.6±0.5°C and an

artificial photoperiod of 10 h light: 14 h dark. All animals were individually housed and each *P. nymphula* larva was supplied with a small piece of plastic drinking straw to act as a perch. Only larvae with three intact caudal lamellae were used in experiments and all were regularly checked for signs of metamorphosis.

When not being used in experiments all animals were fed *ad lib.* with chironomid larvae (experiments 1 and 2) or mosquito larvae, *A. aegypti* (experiments 3 and 4) depending on availability.

Experimental containers were clear, plastic, rectangular tubs measuring 22.5cm × 12cm and 7.5 cm deep, filled with 750ml of copper-free tap water. The tanks were divided in half on the long axis by a partition. The nature of the partition depended on the experimental trial as described below. The edges of the partitions were sealed against the wall of the container with non-toxic plasticine to ensure that the halves were watertight.

Before each experiment, *P. nymphula* larvae were starved for a period of 24 h to standardise hunger levels. All experimental trials commenced at 12.00 and, between trials, containers were cleaned to remove any prey items and chemical odours.

EXPERIMENT 1. – Does the presence of a predator affect foraging activity? Four different trials were set up in the experimental containers. In each case a single *P. nymphula* larva was placed in one half of the container with a perch available and 10 chironomid larvae as prey. The other half of the container housed a single predator, *A. juncea*. Four different partitions were used as below:

Control – An opaque, solid plastic partition separated the two halves of the experimental container allowing no contact.

Visual stimulus – A clear, solid plastic partition separated the two halves allowing only visual contact between the two species.

Chemical stimulus – An opaque partition perforated with 1mm-diameter holes separated the two halves allowing water flow between the two species.

Visual and chemical stimuli – A clear plastic partition perforated with 1mm diameter holes separated the two halves allowing both visual contact and water flow between the species.

The number of chironomid larvae consumed after 24 h was recorded. Each trial was repeated 30 times.

EXPERIMENT 2. – Does the presence of a predator fed on conspecifics affect foraging activity? Experimental containers were set up as before using the clear, perforated plastic partitions with a *P. nymphula* larva and 10 chironomid larvae on one side of the partition and an *A. juncea* larva on the other. The predators' *A. juncea* were fed either one *P. nymphula* larva daily for seven days prior to the commencement of the experiment or supplied with 6-7 chironomid larvae daily. The number of chironomid larvae consumed after 24 h was recorded. The trial was repeated 30 times.

EXPERIMENT 3. – Does hunger level affect foraging activity? In this experiment the hunger level of the *P. nymphula* larvae was manipulated by starving them for different periods before the experiment. A starved *P. nymphula* larva was placed in each side of the experimental container with 6 *A. aegypti* larvae, the two halves of the container were separated by an opaque, solid partition. *P. nymphula* larvae were starved for 0, 24, 48 and 72 h prior to the experiment commencing. The number of *A. aegypti* larvae consumed after 24 h was recorded. Each trial was repeated 10 times.

EXPERIMENT 4. – Do hunger level and chemicals derived from predator combined have an effect on foraging activity? In this experiment the hunger levels of the *P. nymphula* larvae were manipulated as above and the larvae placed in the experimental container. For a week prior to the experiment *A. juncea* larvae were housed in the experimental containers and fed *P. nymphula* larvae. The *A. juncea* larvae and any remaining food traces were removed and the *P. nymphula* larvae introduced. Six *A. aegypti* larvae were offered as prey and the number of these consumed after 24 h recorded.

RESULTS

EXPERIMENT 1: DOES THE PRESENCE OF A PREDATOR AFFECT FORAGING ACTIVITY?

Figure 1 shows the mean number of prey eaten by larvae of *P. nymphula* when exposed to the different experimental stimuli. One-way ANOVA showed a significant difference between the numbers of prey items eaten ($P<0.001$, $F = 15.95$, df = 3,116). A post-hoc Tukey test found no significant difference between the control and visual stimulus and between chemical stimulus alone and visual and chemical stimuli together. Significantly fewer prey were taken in both chemical stimulus alone and visual and chemical stimuli together than in the other two treatments.
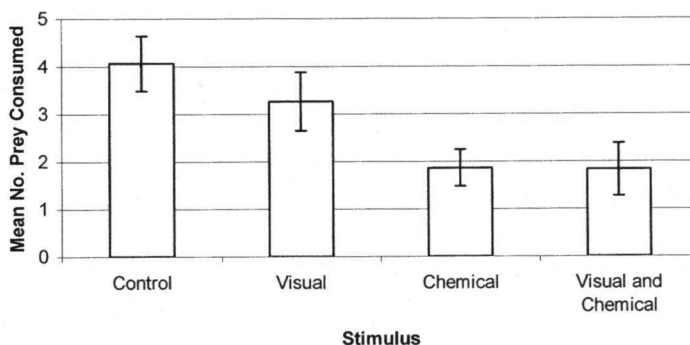
Fig. 1. Mean number of prey eaten by *P. nymphula* larvae under the influence of different predator stimuli. − [Bars show 95% confidence limits]

EXPERIMENT 2: DOES THE PRESENCE OF A PREDATOR FED ON CONSPECIFICS AFFECT FORAGING ACTIVITY?

Figure 2 shows the mean number of prey eaten by larvae of *P. nymphula* when exposed to predators fed on either chironomid larvae or on conspecifics. Foraging activity can be seen to be reduced in the presence of a predator fed on chironomids and then further reduced in the presence of a predator fed on other *P. nymphula* larvae. One-way ANOVA showed a significant difference between the numbers of prey items eaten ($P<0.001$, $F = 60.67$, df = 2,87). A post-hoc Tukey test showed significant differences between all pairs of treatments.

EXPERIMENT 3: DOES HUNGER LEVEL AFFECT FORAGING ACTIVITY?

Figure 3 shows the mean number of prey eaten by larvae of *P. nymphula* after different periods of starvation. One-way ANOVA showed a significant difference be-
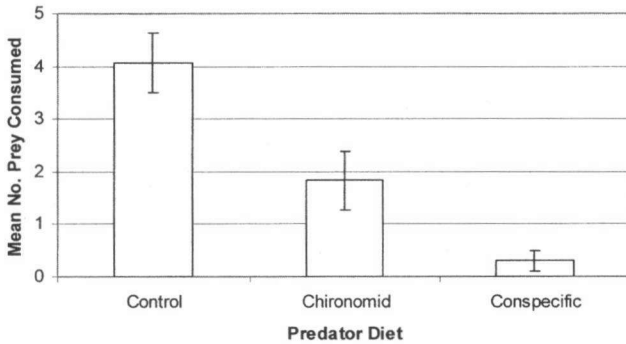
Fig. 2. Mean number of prey eaten by *p. nymphula* larvae under the influence of visual and chemical stimuli from predators fed on different diets. – [Bars show 95% confidence limits]

tween the numbers of prey items eaten after different periods of starvation (P<0.001, F = 92.31, df = 3,156). A post-hoc Tukey test showed no significant difference between the number of prey eaten after 0 and 24 h starvation but significant differences between all other pairwise combinations.

EXPERIMENT 4: DO HUNGER LEVEL AND BEING HOUSED IN WATER CONTAINING CHEMICAL STIMULI FROM A PREDATOR FED ON CONSPECIFICS COMBINED AFFECT FORAGING ACTIVITY?

Figure 4 shows the mean number of prey eaten by larvae of *P. nymphula* after different periods of starvation and in water containing chemical stimuli from a predator fed on other *P. nymphula* larvae. One-way ANOVA showed a significant difference between the numbers of prey items eaten after different periods of starvation (P<0.001, F = 56.94, df = 3,156). A post-hoc Tukey test showed that the number
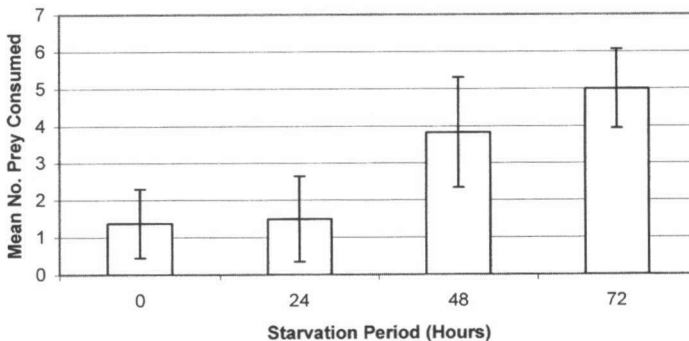

Fig. 3. Mean number of prey eaten by *p. nymphula* larvae after different periods of starvation. ⊠ [Bars show 95% confidence limits]
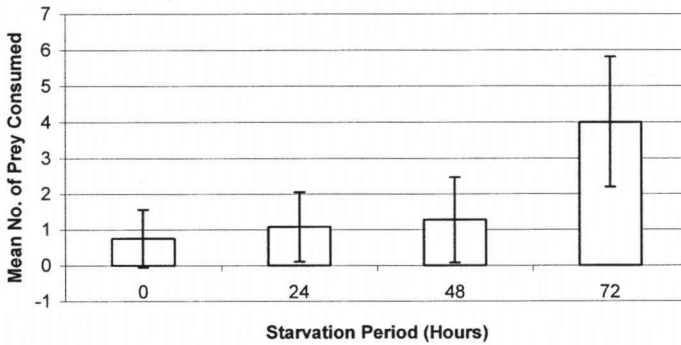
Fig. 4. Mean number of prey eaten by *p. nymphula* larvae after different periods of starvation in the presence of predator fed on conspecific larvae. — [Bars show 95% confidence limits]

of prey eaten after 72 h of starvation was significantly greater than the number of prey eaten at all other starvation levels. There were no significant differences between the numbers of prey eaten at all other starvation levels.

DISCUSSION

The results show that the presence of a predator reduced foraging activity in *P. nymphula*. This agrees with ABRAMS & ROWE's (1996) theory that flexible life cycle species should decrease foraging in the presence of predators and that fixed one year life cycle species would be expected to have high foraging effort (STOKS & JOHANSSON, 2000). As *P. nymphula* is a semivoltine species it can afford to reduce its feeding rate and take longer to develop (STOKS & JOHANSSON, 2000).

Damselfly larvae have long been known to reduce foraging activity in the presence of predators (LIMA, 1998). Predators that impose mortality upon prey are usually detectable to prey and stimulate predator avoidance behaviour. Less is known about the stimuli that cause this response, which may be visual, chemical, mechanical or a combination of these (KOPERSKI, 1997). The results suggest that visual stimuli from predators have no effect in reducing foraging in *P. nymphula*. A similar result was found in a study of *Ischnura elegans* (HEADS, 1986) but STOKS (2001) did find that zygopteran larvae do respond to visual stimuli from predators.

Many studies have demonstrated that prey alter foraging behaviour in response to chemical stimuli from predators. One such study showed that chemical signals induced changes in the feeding rate and diet composition of the damselfly *Enallagma cyathigerum* (KOPERSKI, 1997). The results from the current study suggest that *P. nymphula* are also strongly affected by chemical stimuli from a potential predator. *P. nymphula* larvae ate significantly fewer prey items when exposed to chemical stimuli from a predator than when no predator was present. *P. nymphula* was seen to spend more time on a perch and less time actively foraging than

in control experiments.

Recent studies have shown that chemical signals may be extremely important in aquatic environments, as chemical signals are very often accompanied by predation (VAN BUSKIRK & YUREWISZ, 1998). Other invertebrates show anti-predator behaviour in response to potential predators. Mayfly larvae can chemically detect predatory stonefly larvae and move away from the danger. A similar response has been shown in crayfish (LIMA & DILL, 1990).

The results from the current study suggest no significant reduction in foraging activity from the additive effects of visual and chemical stimuli when compared to chemical alone. Similar results were found by KOPERSKI (1997) in his study on *E. cyathigerum*. An additive effect has been found in other benthic invertebrates (KOPERSKI, 1997) but not as yet in zygopteran species.

There was a clear reduction in foraging activity when *P. nymphula* larvae were in the presence of a predator fed on conspecifics. A wide variety of aquatic animals use chemical cues to assess risk; this may be learned or innate (KORPI & WISENDEN, 2001). Animals that can detect and respond to these cues will have a higher chance of survival as conspecific-released chemical cues are a reliable indicator of predation threat (WISENDEN & MILLARD, 2001). CHIVERS et al. (1996) collected larvae of *Enallagma boreale* and *E. cyathigerum* from a pond that contained fathead minnows but not pike. These larvae were exposed to chemical stimuli collected from pike fed on *Enallagma* spp. larvae, fat head minnows or mealworms. Foraging activity was reduced in the presence of stimuli from the pike fed on *Enallagma* spp. larvae and minnows but not mealworms. This demonstrated that the damselfly larvae were using the predator's diet to assess their own predation risk.

It has been suggested that these behaviours are in direct response to alarm pheromones released by conspecifics rather than by the predators themselves (STOKS, 2001). It is also suggested that recognition of odours as being dangerous is acquired through experience. Recognition has been shown to be easily learned from simultaneous presentation of the odour of a novel predator and a known indicator, such as an injury released chemical cue (WISENDEN & MILLARD, 2001).

P. nymphula larvae were shown to eat more the longer they had been starved. This is an obvious conclusion but has important repercussions for the survival of individuals. The larvae consumed larger numbers of prey items when starved for 48 h compared to 24 h or no starvation. Starving for a further 24 h gave a further increase in consumption of prey.

Food intake was also influenced by the presence of chemical cues from a predator. Only the 72-hour starvation period produced a significant increase in prey consumption. This indicates that the larvae need to reach a higher level of hunger before attempting to forage in the presence of a predator. These results also demonstrate that the physical presence of a predator is not necessary to cause a reaction; chemical cues left behind by a predator are also effective in eliciting a response.

Research into predator prey interactions in damselflies is ecologically important

when an increasing number of species are threatened. Interactions between species can be studied to evaluate the effect they have on each other and to identify if one species threatens another. HEADS (1986) identified a reduction in feeding activity in *Ischnura elegans* in response to its natural predator *Notonecta glauca*. Reduced larval feeding rates can lead to slower growth and development and prolonged instar durations, which may have important consequences for survival and reproductive fitness (HEADS, 1986). JEFFRIES (1990), however, found that, although *E. cyathigerum* significantly reduced movement in response to the dragonfly *A. juncea*, its hunting success was not significantly altered. With so many species breeding in very specific habitats or sites it may become a necessity in the future to take measures to control predators or provide purpose built breeding sites that are free of the significantly harmful predators.

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