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Predator detection and avoidance by lotic mayfly nymphs of different size

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Abstract We studied antipredatory responses of lotic mayfly (*Baetis*) nymphs in a factorial experiment with four levels of fish presence: (1) a freely foraging fish (the European minnow, *Phoxinus phoxinus*), (2) a constrained fish, (3) water from a fish stream, (4) water from a fishless stream. Large *Baetis* nymphs drifted mainly during night-time in treatments involving either the chemical or actual presence of fish, whereas no diel periodicity was observed when the water was not conditioned with fish odour. The response was strongest when the fish was uncaged, which suggests that visual or hydrodynamic cues are needed in addition to chemical ones for an accurate assessment of predation risk. Fish presence had no effect on the drift rates of small nymphs. Instead, they increased their refuge use in the presence of a live fish. Chemical cues alone did not have any effect on the refuge use of any of the *Baetis* size classes. Our results indicate active drift entry by mayfly nymphs. Because predation pressure is spatially and temporally variable, nymphs must sample the environment in order to locate predator-free areas or areas with low predation risk. Drifting should be the most energy-saving way to do this. To avoid the risk from visually feeding fish, large individuals can sample safely (i.e. enter drift) only at night-time, while the small ones can also do this safely during the day. We suggest that, contrary to some earlier assumptions, mayfly drift is not a fixed prey response. Instead, *Baetis* nymphs are able to assess the prevailing predation pressure, and they adjust their foraging behaviour accordingly.

Keywords *Baetis* · Antipredatory responses · Stream Drift · Refuge use

Introduction

Prey animals basically defend themselves in two ways: by completely avoiding predators or by escaping after an encounter with a predator. In order to avoid encounters, prey often change their habitat use, time of activity, and/or overall movement rate. Behavioural avoidance of predators may lead to reduced feeding rate and, ultimately, reduced fitness (Sih 1987; Lima and Dill 1990). Predation risk is typically related to body size, and small prey often enjoy a size refuge from predation (e.g. Allan 1978). In some systems, however, the largest size classes are the least vulnerable ones (Werner and Gilliam 1984; Mittelbach and Chesson 1987; Wahle 1992). Ontogenetic changes in predation risk should select for different antipredatory responses in different prey size classes (Dill 1987).

Downstream drift of stream insects is one of the best documented phenomena in lotic systems (Waters 1972; Brittain and Eikeland 1988). Allan (1978) suggested that the tendency of large individuals of some benthic taxa to drift mainly at night is an antipredatory avoidance mechanism against visually feeding fish. Flecker (1992; see also Sih 1987) suggested that this behavioural trait is genetically fixed, that is, it remains even when fishes are experimentally removed from a stream reach. In streams that historically lack drift-feeding fish, the diel periodicity of drift is often weak or non-existent (Turcotte and Harper 1982; Malmqvist 1988; Flecker 1992).

To avoid encounters with fish, stream invertebrates may also alter their habitat use. This often involves positional changes between a safe refuge and a risky feeding area. Thus, Culp and Scrimgeour (1993) have recently shown that *Baetis* in a fish stream move to the tops of stones to feed at night (see also Kohler and McPeck 1989), whereas *Baetis* from a fishless stream are almost aperiodic in this respect.

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Stream invertebrates may detect fish by visual, hydrodynamic; tactile or chemical stimuli. In freshwater habitats chemical detection of predators is common in tadpoles (Petranka et al. 1987), amphipods (Williams and Moore 1985; Andersson et al. 1986; Andersen et al. 1993) and isopods (Holomuzki and Short 1988). Evidence for chemical detection by insect larvae is sparse, but the activity of stoneflies (Williams 1986; Malmqvist 1992) and possibly caddis-flies (Malmqvist 1992), and substrate use by mayflies (Kohler and McPeck 1989) are affected by fish chemicals.

In this paper we test whether *Baetis* mayfly nymphs (Ephemeroptera: Baetidae) detect fish chemically and alter their behaviour accordingly. By using two *Baetis* size classes, we also examine whether there are any ontogenetic changes in the antipredatory responses of *Baetis* nymphs.

Materials and methods

The experiments were performed at Oulanka Biological Station in northern Finland between 21 September and 4 October 1991. The prey, *Baetis* mayfly nymphs, were collected from a fish stream (mostly burbot and minnow, density 0.10 fish m⁻²). The average head width of large nymphs was 1.46 mm (SD = 0.078, $n = 30$; nymphs with enlarged wing pads excluded), while that of small nymphs averaged 0.79 mm (SD = 0.073, $n = 30$). Species identity of live nymphs could not be confirmed without causing damage to them and, on later inspection, about 80% turned out to be *B. rhodani* and 20% belonged to *B. subalpinus* Ggts. In the laboratory we placed large and small nymphs in separate containers, with ten nymphs per container, and kept them at +7° C for 2 h before the start of an experiment.

The European minnow, *Phoxinus phoxinus* L., was used as a predator. The fish were caught with dip nets in a stream pool, kept in rearing tanks for 3–4 days, and fed with live benthic animals. The evening prior to an experiment, two individuals (mean standard length 60 mm, SD = 10.3, $n = 18$) were transferred to a bucket and kept without food for 16 h.

The glass aquaria used were slightly modified from the model of Soluk and Collins (1988) (Fig. 1). Flow in an aquarium was maintained with air pumped through a diffuser. Water was recirculated in each aquarium, and animals drifting downstream inevitably re-entered the arena. Current velocity near the bottom was 15 cm s⁻¹, which is within the range of velocities experienced by *Baetis* in the wild (P. Tikkanen, personal observation). A dense mesh cloth was fastened with silicon sealant to the bottom of the arena to provide a foothold for the nymphs. Eight rough-surfaced tiles (49 mm×49 mm×5 mm) were arranged in two rows parallel to the flow. The tiles were raised from one edge by a shallow support so that we could readily observe *Baetis* on all tile surfaces. Before an experiment, tiles had been incubated in a nearby fishless stream for 2 weeks, and a visible periphyton layer had developed on the upper surface of the tiles. *Baetis* nymphs easily maintained their position on all surfaces, and grazed algae on the upper sides of the tiles. The lower tile surfaces and the arena beneath the tiles could not be reached by the minnow, and thus this space was regarded as refuge free from predation. Thirty five per cent of the projected area was tile surface containing food for *Baetis*.

Our experimental set-up was factorial, with fish presence (four levels) and time of day (three levels) as factors. Each treatment was replicated 7 times. Due to practical limitations, we used the same set of nymphs during all observations within 1 day. Therefore, the design is one of repeated measures. Ten large and ten small nymphs were observed simultaneously in an aquarium and, therefore, *Baetis* size classes cannot be regarded as truly independent factor levels. However, we did not observe any interactions

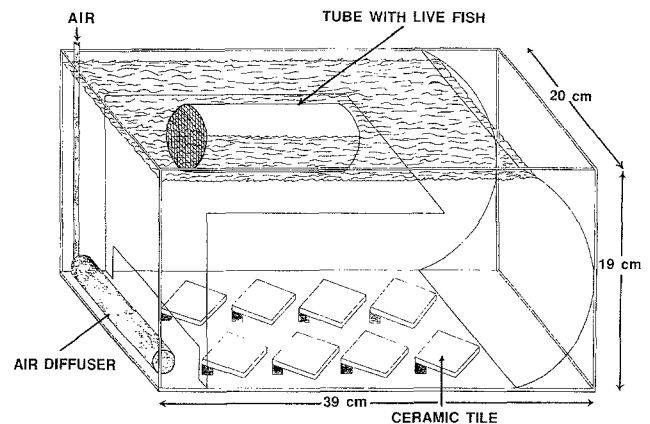


Fig. 1 Glass aquarium used in the experiments. A recirculating flow passing through the tube containing a live minnow was produced by the air diffuser

between nymphs of different size. Thus, unless otherwise stated, *Baetis* size groups were treated separately in statistical analyses.

The presence or absence of minnow was simulated as follows: (1) water from a fishless stream ("fishless stream"), (2) water from a stream with a rather high fish density (0.9 individuals m⁻², "fish stream"), (3) water from a fish stream together with the odour of one minnow ("fish odour") and (4) water from a fish stream together with one minnow swimming freely in the aquarium ("free minnow"). Fish odour was provided by keeping a live minnow in an opaque plastic tube (diameter 5 cm) with netting at both ends. The tube was placed parallel to the flow on the horizontal baffle (see Fig. 1), so that the minnow could not be seen by *Baetis* nymphs. To prevent contamination between days, fish presence treatments were run in separate aquaria. To control for a possible position effect in relation to illumination, we changed the positions of aquaria clockwise every day. The experiments were run in an outdoor building, where daylight entered the room from open doors, and illumination was similar to that of a shaded stream.

The water and tiles were changed every morning, and *Baetis* nymphs added to the aquaria around noon. Ten large and ten small *Baetis* were allowed to settle in the arena in standing water, whereafter water flow was slowly increased. We made behavioural observations 3 times a day, during the daytime (1400–1600 hours, "day"), around dusk (1800–2000 hours, "dusk"), and at night (2130–2330 hours, "night"). During dusk and night periods, a dim red light was used to facilitate observations.

In the beginning of a 30-min observation period, we recorded the positions (refuge versus exposed) of all nymphs in an aquarium. During the next 15 min, we noted all drift entries (defined as entry into the water column), positioning changes, and, in treatments with a free minnow, direct escapes following an encounter with the fish. Following this, we noted the foraging activity of three focal individuals per size class, for periods of 1 min each. We used the number of movements (defined as changes in position) as an indication of a nymph's feeding rate (see also Kohler and McPeck 1989). At the end of an observation period, another "snapshot" of the positions of the nymphs was made. This whole procedure, which lasted for about 30 min, was repeated in all four aquaria. In the "free minnow" and "fish odour" treatments, the fish was introduced immediately before, and removed immediately after, an observation period. After "night" observations, we collected and preserved the nymphs, and emptied and thoroughly cleaned the aquaria. To examine whether algal food had been depleted, we scraped periphyton from the upper and lower sides of 3–4 randomly selected tiles per aquarium into 200 ml distilled water. The samples were kept in the dark for 7–8 h, and then filtered through a fibreglass filter (Whatman GF/C). Chlorophyll *a* was measured spectrophotometrically. As a control, we also sampled upper and

lower surfaces of ten ungrazed tiles, which were kept in a holding tank similar to the experimental arena but without grazers.

Data analysis

The null hypothesis of no difference in drift entries between treatments was tested with a repeated measures analysis of variance (PROC GLM, SAS Institute Inc 1989). To reduce heteroscedasticity (shown by Bartlett's test and correlation between means and variances), numbers of drift entries were logarithmically transformed. Variances of changes in drift entries across the repeated measurements (here, drift entries in "day", "dusk", and "night") should be equal if the univariate mode of repeated measures ANOVA (ANOVAR) is to be used (LaTour and Miniard 1983; Crowder and Hand 1990). In our drift data, Mauchly's test for sphericity and Huynh-Feldt criterion showed that the condition of equal variances was not met. Therefore, we used the multivariate mode of ANOVAR (MANOVAR; for selecting the appropriate method in ANOVAR, see Potvin et al. 1990).

At the time of the year when the experiment was conducted, darkness fell during the "dusk" period. To calculate the night/day ratio (N/D) of nymphs entering the drift, we assigned the first 15 min of "dusk" observations to day, the last 15 min to night, and excluded the remaining ones. N/D ratios greater than 1 (a one-tailed test) indicate nocturnally biased drifting. To test whether the departure from 1 was significant, we randomly assigned the observed drift counts to either day or night, and recalculated the ratio. This procedure was repeated 10 000 times for each fish presence level using a BASIC computer program. The *P*-value for rejecting the null hypothesis (N/D ratio not significantly greater than 1) is the proportion of randomized ratios (out of 10 000) more extreme than the one observed (see Crowley 1992).

We also analysed the change of refuge use by *Baetis* (the decrease or increase in numbers of nymphs using refuges during an observation period) with ANOVAR. Transformation of the data was not needed (moderate normality, homoscedasticity) and, since variances of changes across time of day were rather homogeneous (Huynh-Feldt criterion), the adjusted univariate mode of ANOVAR was used (see LaTour and Miniard 1983; Crowder and Hand 1990).

Differences between the four fish presence/absence levels with respect to the feeding activity of nymphs were analysed with a 2-way ANOVA. A full factorial design could not be applied because there were too few observations in some treatments. Therefore, the data from all time periods were pooled for this analysis.

Results

Water temperature in the aquaria ranged from 3 to 8°C, depending on the ambient temperature. Within this narrow range, there were no significant correlations between temperature and the response variables. Neither was there any indication of food depletion during the trials, nor of any difference in the amount of periphyton between the fish presence levels and the ungrazed control (Table 1). Therefore, we reject the possibility that diel temperature variation or periphyton depletion affected the behaviour of *Baetis* nymphs in our experiments.

The upper surfaces of tiles supported a relatively high standing crop of periphyton ($\bar{x} = 5.4 \mu\text{g chl } a \text{ cm}^{-2}$, $SD = 1.9$, $n = 32$), while the amount of algae on the lower surfaces was negligible ($\bar{x} = 1.0 \mu\text{g cm}^{-2}$, $SD = 0.3$, $n = 32$). Accordingly, nymphs that entered a refuge upon detecting a predator very likely suffered a cost in terms of reduced food intake. This is further supported by the fact

Table 1a The amount of periphytic algae ($\mu\text{g chlorophyll } a \text{ cm}^{-2}$) on the upper and lower surfaces of tiles; **b** two-way ANOVA table

	Upper tile surface			Lower tile surface		
	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>
Fishless stream	5.62	1.62	8	1.00	0.33	8
Fish stream	4.96	1.71	8	0.96	0.21	8
Fish odour	5.08	1.67	8	0.96	0.33	8
Free minnow	5.79	2.71	8	1.00	0.29	8
Ungrazed control	4.54	1.68	10	1.04	0.64	10

b)			
Source of variation	<i>F</i>	<i>df</i>	<i>P</i>
Fish presence/absence	0.546	4	0.702
Surface (upper and lower)	358.9	1	0.000
Fish presence × surface	0.498	4	0.737

that nymphs on the lower surfaces were never observed feeding.

Drift behaviour

The overall drift propensity of large nymphs (mean number of drift entries $15 \text{ min}^{-1} = 3.7$, $SE = 0.51$) was higher than that of small nymphs ($\bar{x} = 1.4$, $SE = 0.29$). In MANOVAR, time of day was the only significant main effect for small *Baetis* (Table 2). Although the nymphs drifted more at night (mean number $15 \text{ min}^{-1} = 1.74$, $SE = 0.56$), drift during daytime was almost as frequent ($\bar{x} = 1.5$, $SE = 0.59$). The fish×time interaction term was not significant. However, the generally low drift activity of small nymphs may have obscured the analysis. In 59% of the trials small nymphs did not drift at all.

For large nymphs the main effects of time of day and fish presence were both significant. More importantly, the fish×time interaction was also marginally significant (Table 2). This resulted mainly from the tendency of large nymphs to drift during night-time in the "free minnow" treatments (Fig. 2).

Some drift entries in the "free minnow" treatment appeared to be direct escapes after a close encounter with fish. The number of escape driftings was equal across time periods, and thus, the predominantly nocturnal drift also remained if escaping nymphs were excluded (Fig. 3). No attempt was made to quantify the encounter frequency between minnows and *Baetis* nymphs. Instead, the activity of the minnows was subjectively scored on an ordinal scale (0–5). A sign test showed that the minnows were equally active throughout the day ($M = 2$, $n = 7$, $P = 0.227$). Therefore, we assumed that encounter frequency was constant across time periods, and that the increased nocturnal drift of large *Baetis* did not result from an increased number of encounters with the minnow.

N/D drift ratios of small nymphs did not differ significantly from 1. For large nymphs, this ratio was almost

Table 2a,b Two-way repeated measures analysis of variance (multivariate mode) for the effects of time of day and fish presence on the number of drift entries by *Baetis* nymphs during 15 min **a** large *Baetis*, **b** small *Baetis*

Source of variation	Pillai's trace	F	df	P
a)				
Within-subjects				
Time	0.607	17.76	2,23	0.000
Time*fish	0.443	2.27	6,48	0.051
Between-subjects				
Fish		5.17		0.007
b)				
Within-subjects				
Time	0.548	13.96	2,23	0.000
Time*fish	0.096	0.40	6,48	0.874
Between-subjects				
Fish		0.31		0.819

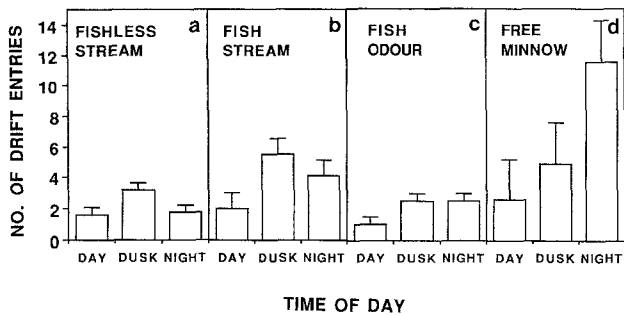


Fig. 2 Number of drift entries by large *Baetis* nymphs per 15 min at different times of the day (mean \pm SE, $n = 7-8$). **a** Water from a fishless stream; **b** water from a fish stream; **c** water from a fish stream plus a caged minnow; **d** water from a fish stream plus a free minnow

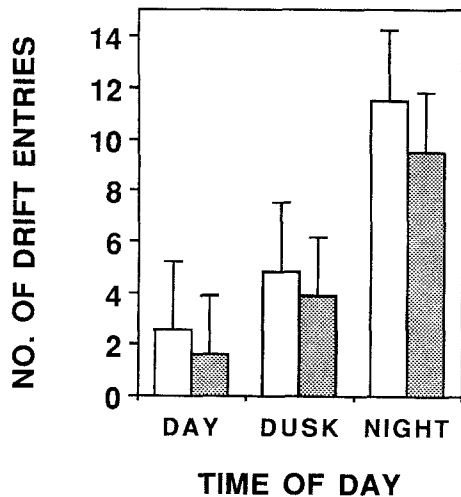


Fig. 3 Number of drift entries by large *Baetis* nymphs per 15 min in the "free minnow" treatments (mean \pm SE, $n = 7-8$). (open bars = all drift entries, hatched bars = escape entries excluded). The number of escape drift entries was equal across the day (one-way ANOVA, $F_{2,12} = 0.062$, $P = 0.94$).

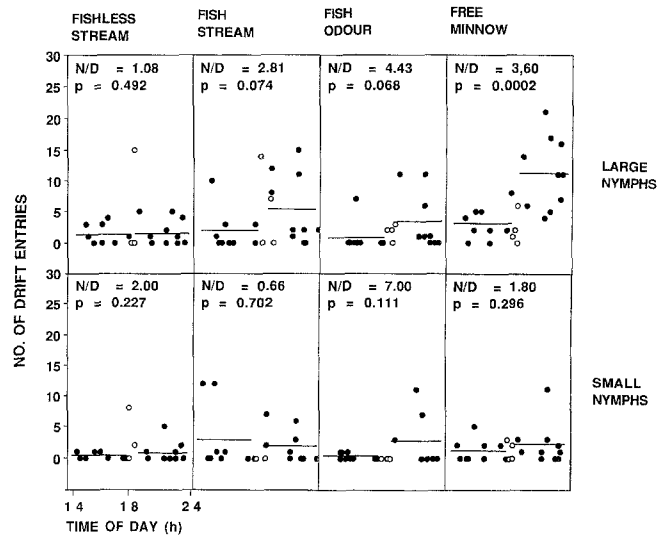


Fig. 4 Number of drift entries by large and small *Baetis* nymphs per 15 min in the four fish presence/absence treatments in relation to the time of day. Open dots indicate observations excluded from the calculation of night/day ratios (N/D). Details for excluding some observations and the method of calculating the probability value (P) are given in the text. Horizontal lines denote mean number of drift entries in day and night observations

Table 3 a The effect of fish presence/absence (four levels) on the foraging activity (the number of feeding movements per 1 min) of small and large *Baetis* nymphs; **b** two-way ANOVA table

a)

Fish presence/absence	No. of movements			
	Large		Small	
	x	SE	x	SE
Fishless stream	3.21	0.67	2.36	0.40
Fish stream	3.68	0.69	2.74	0.73
Minnow odour	3.57	0.75	2.53	0.51
Free minnow	2.93	0.88	1.31	0.33

b)

Source of variation	F	df	P
Fish presence	0.94	3	0.423
Size	5.61	1	0.020
Fish presence \times size	0.81	3	0.948

one in "fishless stream" treatments, whereas in other fish presence treatments it was considerably greater (Fig. 4). The presence of uncaged fish ("free minnow") caused large nymphs to postpone their drift until darkness, and, interestingly, the same trend was also observed in treatments involving the mere chemical presence of fish ("fish stream", "fish odour") (Fig. 4).

Feeding activity and refuge use

There were no differences among fish presence levels in the feeding activity of *Baetis* (Table 3). The nymphs

Table 4 Two-way repeated measures analysis of variance for the effects of time of day and fish presence on refuge use by: **a** large *Baetis* nymphs and **b** small *Baetis* nymphs. Refuge use was measured as a change in the number of nymphs in refuge during 15 min of observation. Significance levels adjusted with the Huynh-Feldt epsilon criterion

Source of variation	<i>F</i>	<i>df</i>	<i>P</i>
a)			
Within-subjects			
Time	0.38	2	0.686
Time*fish	0.26	6	0.951
Between-subjects			
Fish	0.49	3	0.690
b)			
Within-subjects			
Time	1.07	2	0.350
Time*fish	0.37	6	0.894
Between-subjects			
Fish	5.67	3	0.004

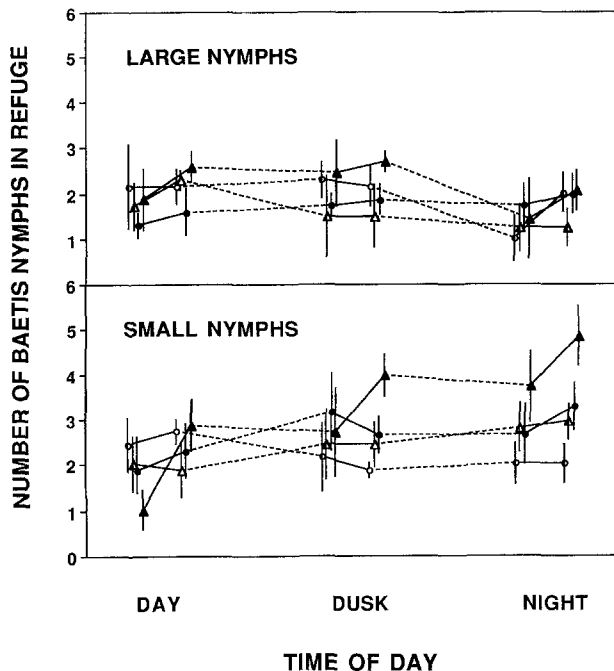


Fig. 5 Number of large and small *Baetis* nymphs in refuges (mean±SE) in the four fish presence levels at different times of the day. *open circle* = “fishless stream” water, *open triangle* = “fish stream” water, *filled circle* = “fish odour” present, *filled triangle* = “free minnow” present. *Solid lines* denote changes in the number of nymphs in refuges during an observation period, and *dashed lines* denote changes in the number of nymphs in refuges between two observation periods, (with the fish removed in “fish odour” and “free minnow” treatments). For small nymphs, refuge use in the “free minnow” treatment was significantly different from other fish presence levels (one-way ANOVA for data pooled over time levels, $F_{3,24} = 5.51$, $P = 0.002$, followed by Tukey’s test)

tended to be less active in the “free minnow” treatments, but this trend was not significant.

On average, about 20% (two out of ten) of the nymphs in both size classes settled initially underneath

the tiles (Fig. 5). Large nymphs did not change their refuge use in any of the treatment combinations (Table 4). In contrast, the effect of fish presence was highly significant for small *Baetis* nymphs. This was caused by increased refuge use in the presence of a freely foraging minnow. Out of eight small nymphs initially exposed, one or two entered the refuge per observation period (Fig. 5). Increased refuge use by small nymphs in the presence of a freely swimming fish occurred throughout the day, as indicated by an insignificant time×fish interaction term (Table 4). Consequently, by the end of the day (after three 30-min exposures to fish) 50%, on average, of the small nymphs were beneath the tiles. It is important to notice that this prey response was immediate. Nymphs entered the refuge only when exposed to a live fish, and no additional entries were observed between observation periods when the fish was removed from the aquarium (Fig. 5). In treatments merely with chemical cues of fish, neither small nor large nymphs altered their refuge use patterns.

Discussion

Drift mechanisms

The mechanisms of drift entry by benthic invertebrates have been debated for more than 20 years. The passive entry hypothesis (Elliott 1968) suggests that drift is mainly accidental, because epibenthic activity makes insects susceptible to dislodgement by flow. If foraging activity on the tops of stones is highest at night, peak drift will also occur at night. However, in direct field observations, Allan et al. (1986; see also Wilzbach 1990) did not find any correspondence between drift rate and epibenthic density and activity of *Baetis* nymphs. Furthermore, Poff et al. (1991) showed that the diel size distribution of drifting *Baetis* nymphs remained unaltered following stream flow manipulation during the day, suggesting that nymphs have behavioural control over their drift entry. Kohler (1985) suggested that drift might serve an important adaptive function in the foraging behaviour of *Baetis* nymphs. Thus nymphs actively enter drift after a resource patch has been depleted below some threshold level. Being at the greatest risk of fish predation, the largest nymphs postpone drifting until darkness, when visually hunting fish are inefficient.

Our results give further support to the active entry hypothesis. The number of large *Baetis* nymphs on exposed surfaces did not differ between times of day, yet the nymphs drifted mainly during night-time. Moreover, the relatively low flow rates in our aquaria did not seem to cause any problems for the nymphs in holding their positions on the tile surfaces. In other studies, large *Baetis* nymphs have been shown to retain their positions in currents well over 50 cm s⁻¹ (Corkum et al. 1977).

In the absence of food depletion, nymphs might also be expected to leave a patch in search of more profitable ones. For example, black fly larvae often abandon their

feeding sites, yet an individual is incapable of depleting its food resource (D. Hart, personal communication). However, we suggest that the nocturnal drift of large nymphs in our experiments was not linked to any food-finding behaviour. Instead, in treatments where fish cues were present, the nymphs perceived a continuous risk of predation. The predation regime in natural streams is variable in time and space (e.g. Sih 1992), and it is important to potential prey to gain information about relative risks in different microhabitats. Potential prey leave patches of the stream bottom with high predation pressure and actively search for safer patches (see also Dill 1987). For efficient swimmers such as *Baetis* nymphs, drifting should be the most energy-saving way to do this. Entering the water column during daytime in the presence of visually hunting fish is, however, dangerous for large nymphs, and they drift predominantly under the cover of darkness (cf. Allan 1978, 1984). Thus, the large nymphs in particular will actively enter the drift in order to locate predator-free areas or areas with relatively low predation risk. It must be emphasized that drift-entering for the search of predator-free areas and/or the location of unexploited food patches are not exclusive. Indeed, we assume that they often act in concert.

Drift can also be initiated as a direct escape from an approaching predator (e.g. Peckarsky 1980; Wiley and Kohler 1981, Malmqvist and Sjöström 1987; Lancaster 1990). If the predator is active by night and, especially, if it selectively encounters/attacks large prey, this could clearly be an important mechanism causing drift periodicity of large *Baetis* nymphs. Recently, Culp et al. (1991) documented a 30-fold increase in the drift rate of *Paraleptophlebia* mayfly nymphs shortly after a nocturnal predator, the longnose dace, was introduced into a laboratory stream. Although the overall activity of minnows in our experiments seemed rather constant across time periods, another study has shown that the European minnow is a diurnal, visually hunting predator (Müller 1973). Accordingly, the nocturnal peak in the drift of large nymphs should not have resulted from an increased number of encounters with fish at night. In fact, the number of drifting nymphs resulting from direct escapes was nearly constant across the day. Nocturnal drift peak was also observed in treatments where direct encounters between the predator and the prey were not allowed.

Chemical cues and size-specific risk

We found evidence for nocturnally constrained drifting of large *Baetis* in response to chemical cues of fish. Chemical detection of fish is known to occur in other fishes (Keefe 1992), crustaceans (Holomuzki and Short 1988; Andersen et al. 1993), and especially in amphibians (Petranka et al. 1987; Sih et al. 1992). In some studies, insect larvae have also been shown to alter their behaviour when exposed to chemical signals of a predator. Thus, stonefly nymphs either increase or decrease their activity (Williams 1986), *Baetis* mayfly nymphs change

their microhabitat (Kohler and McPeck 1989), and *Agapetus ochripes* caddis larvae reduce their movement rate (Malmqvist 1992). However, results are not unequivocal, and some lotic insects have been found not to respond to fish odour (e.g. Williams 1990; Malmqvist 1992).

The response of large *Baetis* nymphs was strongest when the predator was allowed to move freely in the aquarium. This suggests that hydrodynamic and/or visual cues from a freely moving fish were perceived as increased predation risk. Fish odour may be enough to trigger an avoidance response (i.e. drifting in darkness), but additional cues are required for a more accurate assessment of the prevailing predation risk. In contrast, and in concert with Allan's (1978) drift periodicity hypothesis, small *Baetis* nymphs did not alter their drift behaviour in response to the presence of a fish. Instead, they increased their refuge use, but only when the risk of encountering an actively foraging fish was high. Since practically no food was available on the lower stone surfaces, nymphs that abandoned the risky but rewarding microhabitats on the tops of stones very likely suffered a cost of reduced feeding. Interestingly, the nymphs did not return to the upper surfaces between the periods of exposure to fish. In a study of refuge use by *Paraleptophlebia* nymphs, Culp et al. (1991) also observed a considerable time lag before the nymphs returned to the top surfaces of stones after an encounter with fish. Nymphs hiding in refugia may be uncertain about the prevailing predation risk (see Sih 1992), and sampling the predation climate may be too risky a business. Regardless of fish presence, large nymphs remained on the upper surface of stones. While feeding on periphyton on stones, large *Baetis* nymphs seem willing to accept some risk (i.e. they are "risk reckless", sensu Fraser and Huntingford 1984), which they can afford to be by virtue of their well-developed escape abilities. Large *Baetis* nymphs are more efficient swimmers than their smaller conspecifics (Malmqvist and Sjöström 1987; Allan and Feifarek 1989). Thus, for large nymphs, staying in refugia in the presence of drift-feeding fish should apparently be an overestimate of risk (see Bouskila and Blumstein 1992).

Drift as a flexible avoidance response

If an antipredatory trait is not too costly in terms of individual fitness, it should become fixed by natural selection (Stein 1979). In the case of diel drift periodicity the costs are obvious: postponing drift until darkness in the absence of fish should mean an unnecessary confinement to patches with low food availability. Reduced food intake and, ultimately, lowered individual fitness should be the consequences of prolonged visits to unprofitable feeding patches (Holomuzki and Short 1988; Feltmate and Williams 1991). In our experiments, large *Baetis* nymphs did not show diel drift periodicity in treatments devoid of the cues of fish presence. However, when fish chemicals were present they drifted nocturnally. This

suggests that large nymphs were able to assess the risk of predation quite accurately and adjust their behaviour accordingly. Fish exclusion experiments in streams incorporate many confounding factors, which make them hard to evaluate. Thus, in Flecker's (1992) study, trout were excluded from small cages, and yet no changes were observed in the diel drift periodicity of *Baetis* nymphs. He interpreted this as evidence for a fixed behavioural pattern. However, as also pointed out by Flecker (1992) himself, fish exclusion does not exclude effects from chemical exudates from other fishes outside the cages (see also Allan 1982).

Mayfly nymphs in historically fishless streams often drift aperiodically (Turcotte and Harper 1982; Malmqvist 1988; Flecker 1992), indicating that fish are responsible for drift periodicity. Do *Baetis* nymphs hatched and grown in fishless streams perform immediate behavioural adjustments when exposed to fish? In a laboratory experiment on diel periodicity of microhabitat use by *Baetis bicaudatus* Dodds nymphs from a fishless stream, Cowan and Peckarsky (1994) showed that the nymphs adopted a nocturnal activity pattern when exposed to water from a trout stream. However, this study did not address drift periodicity per se.

Although present knowledge concerning adult dispersal between stream insect populations is scanty at best (e.g. Jackson and Resh 1992; Robinson et al. 1992), within a region it seems unlikely that populations in fishless streams are genetically isolated from populations in streams with fish. This conclusion is supported by a study where the aerial colonization of a virgin stream channel was followed (Malmqvist et al. 1991). The initial phase of colonization by *Baetis* mayflies was very rapid because of adult immigrants from adjacent streams. Similarly, Wallace et al. (1986) documented rapid colonization of headwater streams after a complete insecticidal elimination of aquatic invertebrates. Thus, fixed behavioural patterns in immature stages of any population inhabiting a certain stream are not likely to evolve when the adults move between streams.

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