Nocturnal drift of mayfly nymphs as a post-contact antipredator mechanism

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SUMMARY

1. The predominantly nocturnal constrained drift of stream invertebrates is commonly regarded as a behaviour that avoids encounters with visually foraging fish in the water column. The alternative explanation, that drift peaks are caused by bottom-feeding, nocturnal predators, has rarely been tested.

2. We examined these hypotheses by collecting invertebrate drift in five streams in northern Finland: one with brown trout (*Salmo trutta*, a drift-feeding fish), one with alpine bullhead (*Cottus poecilopus*, a benthic fish), one with both species, and two fishless streams.

3. Drift by *Baetis* mayflies was aperiodic or slightly diurnal in both fishless streams on all sampling occasions. In contrast, drift was nocturnal in streams with trout and, to a lesser extent, in the stream with bullhead. Non-dipteran prey drifted mainly nocturnally in all streams with fish, whereas Diptera larvae were less responsive to the presence of fish.

4. In laboratory experiments, bullheads were night-active, causing a much higher frequency of drift by touching *Baetis* at night than during the day. Thus, increased nocturnal drift may serve to avoid both visual predators (a pre-contact response) and benthic fish (a post-contact response). In streams with bottom-feeding fish, nocturnal drift should be caused by increased drift by night rather than by reduced drift by day.

Keywords: alpine bullhead, diel periodicity, macroinvertebrate drift, mayfly nymphs, predation risk

Introduction

The nocturnal peak in drift of lotic invertebrates has received considerable attention among aquatic ecologists. It was previously believed that nocturnal drifting is a reflection of diel vertical movements of benthic invertebrates: numbers on stone tops would peak at night and, since individuals on exposed microhabitats are vulnerable to accidental dislodgement, drift catches would peak by night (Elliott, 1968). More recently, explanations emphasizing active behaviour by invertebrates, especially the avoidance of visually hunting, day-active fish (Allan, 1978), have gained acceptance. Invertebrates use drifting as a low-cost means of locating unexploited food patches or areas with low predator density (Kohler, 1985; Tikkanen, Muotka & Huhta, 1994; Forrester, 1994) and, because drift-feeding fishes are size-selective, the largest prey individuals drift most in darkness. Drift of smaller individuals, in contrast, is aperiodic (Allan, 1984; Kohler, 1985). Obviously, the 'accidental' and 'behavioural' hypotheses are not mutually exclusive, and both mechanisms may often work in concert to induce nocturnal peaks in macroinvertebrate drift density.

Hypotheses invoking the avoidance of predators emphasize the role of visual predators and 'pre-con-

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tact' mechanisms to evade them. Non-visual predators, including many benthic fish that rely on tactile cues for prey detection (Hoekstra & Janssen, 1986; Culp, Glozier & Scrimgeour, 1991), are much less studied. There is, however, some evidence that bottom-feeding fish can induce nocturnal peaks in drift density. Culp *et al.* (1991) observed a 30-fold increase in the drift rate of *Paraleptophlebia* sp. mayfly nymphs in the presence of a benthic, night-active fish, the longnose dace (*Rhinichthys cataractae* Valeciennes). In New Zealand, McIntosh & Townsend (1994) showed that movements of the native river galaxias (*Galaxias vulgaris* Stokell) dislodged *Nesameletus* mayfly nymphs from the substratum, leading to a nocturnal increase in drift. It thus appears that nocturnal drifting may serve to avoid visual predators (by a pre-contact response) or tactile predators (by a post-contact response).

We examined the effects of drift-feeding versus bottom-feeding fish on the periodicity of stream drift using a comparative approach ('a natural experiment' *sensu* Diamond, 1983). We collected invertebrate drift in five streams in northern Finland: one with brown trout (*Salmo trutta* (L.); a drift-feeding fish), one with alpine bullhead (*Cottus poecilopus* Heckel; a bottom-feeding fish), one with both fish species, and two fishless streams. Our hypothesis was that if benthic fish do not cause nocturnal drifting, drift periodicity in the stream with alpine bullheads should not differ from that in the two fishless streams. Most previous comparative studies (Malmqvist, 1988; Flecker, 1992; Douglas, Forrester & Cooper, 1994) have included only fishless streams and streams with drift-feeding fish and thus cannot distinguish between the two drift-releasing mechanisms. Finally, we made detailed observations on the behavioral interactions between bullheads and mayfly nymphs in laboratory trials, where *Baetis* mayflies were exposed to freely foraging fish.

Methods

Study sites

The field study was conducted at five sites in the Oulankajoki river system, north-eastern Finland. Four of the sites are small headwater streams (width: 3–6 m) and one is a third-order tributary (width: 25 m) of the River Oulankajoki. The streams are similar in their physical structure. They all have cobble and boulder substrata (median particle size of 7 according to a modified Wentworth scale; see Mäki-Petäys, Muotka, Huusko, Tikkanen & Kreivi, 1997), water velocity ranging from 0.3 to 0.8 m s^{-1} and depth ranging from 0.1 to 0.3 m. Fish density was calculated from repeated electrofishings in the years 1992–1994. At least three separate sections, each covering 120–150 m^2, were electrofished in each stream, one to three times per year. Each sample consisted of three passes using a backpack electroshocker; no block nets were used to isolate the sections. The only fish species in the Putaanoja stream was the alpine bullhead (also known as the eastern sculpin) with a mean density of 0.50 m^{-2}. The Merenoja stream contained brown trout (0.35 m^{-2}) and some European minnow (*Phoxinus phoxinus* (L.); < 0.1 m^{-2}), both of which feed from the drift (McIntosh & Townsend, 1995; Tikkanen *et al.*, 1994). The fish assemblage in the Kuusinkijoki river was more diverse, consisting of five species, with brown trout (0.45 m^{-2}) and alpine bullhead (0.80 m^{-2}) as dominants making up approximately 85% of the fish fauna. The absence of fish from the Juhtipuro (hereafter fishless stream 1) and Uopajan-puro (fishless stream 2) streams was confirmed by extensive electrofishing (covering *c*. 400 m^2 in each stream) conducted in three successive years in both streams. In these streams, a series of waterfalls have presumably acted as barriers for the upstream migration of fish.

Benthic samples (Surber samples, area 0.1 m^2, mesh size 0.25 mm, $n = 12$–15 per stream) were collected to estimate the density of predatory invertebrates (mainly perlodid stonefly nymphs and *Rhyacophila* spp. caddis larvae) in each stream. Density was highest in the two fishless streams (fishless stream 1: mean 184, range 40–310 ind m^{-2}; fishless stream 2: mean 260, range 70–490 ind m^{-2}). A relatively high density was also recorded for the stream containing both fish species (Kuusinkijoki; mean 140, range 70–210 ind m^{-2}) and the stream with bullhead only (Putaanoja; mean 120, range 70–190 ind m^{-2}), while density in the trout stream Merenoja was much lower (mean 49, range 3–88 ind m^{-2}).

Drift sampling

We sampled drift over 24-h periods on three separate occasions: 1–5 July, 6–12 August and 21–29 Septem-

ber 1992 (except for fishless stream 1, where drift was sampled on 1–2 July, 8–9 August and 27–28 September, 1994). Eight 1-h samples were collected at 3-h intervals over each 24-h period. In each stream, we chose a 30–40-m long riffle section for sampling. Four to six stationary drift samplers (plastic tubes fitted with a 1-m long net: mouth, 80 cm²; net mesh size, 0.25 mm) were operated simultaneously. To calculate the volume of water sampled by each net, we measured the current velocity at the net mouth after each collection interval. Drift density of macroinvertebrates (numbers m^{-3}) was calculated by dividing the number of animals in the net by the volume of water filtered. It should be noted that, due to clogging of the nets, the final current velocity measurements are usually lower than those measured at the start of a sampling interval. Since we used the final value (no start value was measured), our estimates of drift density may be too high. However, since these are oligotrophic, clearwater streams and our sampling interval was relatively short (1 h), clogging of the nets was negligible, and we consider this bias to be insignificant. Drift samples were preserved in 70% ethanol, and invertebrates were later sorted in the laboratory and identified to the lowest feasible taxonomic level (usually species). Most analyses focused on *Baetis* nymphs (Ephemeroptera), because these were always abundant in the drift, and they were one of the major constituents of the fish diet in our study streams. Pooling of different *Baetis* species (three to five per stream) seemed justified, because about 90% of all *Baetis* nymphs were either *Baetis rhodani* Pict or *Baetis subalpinus* Bgtss, whose morphology and habitat requirements are similar (Ulfstrand, 1967). The head widths of *Baetis* nymphs were measured to the nearest 0.1 mm using a dissecting microscope fitted with an ocular micrometer. Five Surber samples were taken from each site after drift sampling was completed.

The simultaneous collection of drift and benthic samples allowed us to estimate the instantaneous probability of a *Baetis* nymph entering the drift, using the method of Elliott (1967):

$$P(\text{drift}) = \frac{(\text{drift density})(\text{water depth})}{(\text{benthic density}) - (\text{drift density})(\text{water depth})}$$

where P(drift) is the instantaneous probability of an individual entering the water column, also considered as the instantaneous proportion of population drifting (Elliott, 1967). To isolate the mechanism causing alterations in the diel drift activity (an increase in night-time drifting versus reduction in daytime drifting), we calculated drift probability separately for the day and the night samples. In July, only the sample started at 00:00 was considered to represent night drift, and the mean of drift density at other sampling intervals was used to calculate day P(drift). Samples started at 00:00–03:00 in August and 21:00–03:00 in September were assigned to night drift. The mean night drift density was then used to obtain night P(drift), and day P(drift) was calculated using the mean of the daytime samples. Drift probability was also calculated for two other prey groups: dipteran larvae (mainly blackfly larvae and midge larvae), and a mixed group hereafter called 'other prey', which consisted of mayflies other than *Baetis*, stonefly (Plecoptera) nymphs and caddis (Trichoptera) larvae.

Both salmonids (Allan, 1978; Newman & Waters, 1984) and sculpins (Newman & Waters, 1984; Englund & Olsson, 1996) are size-selective predators. We therefore examined whether diel differences in drift were size-specific by calculating the night/day (N/D) drift density ratios for three size classes (head width < 0.45 mm, 0.45–0.75 mm, > 0.75 mm) of *Baetis*. Drift collections were assigned to either 'day' or 'night' samples as previously done for drift probabilities. We used a binomial test (Sokal & Rohlf, 1981) to examine the null hypothesis that N/D ratios did not deviate from unity, i.e. the probability of drifting during the day and the night was equal. Because of the large number of tests conducted, we maintained the experiment-wise risk level at 0.05 for each sampling date by applying a Bonferroni correction, the corrected risk level being 0.05 per number of comparisons on a particular date. To provide an overall test for the hypothesis, we first calculated a combined N/D ratio for the two largest size classes and tested for its deviation from 1.0, separately for each stream and sampling date. We then used Fisher's combined probability test (Sokal & Rohlf, 1981) to test for an overall trend (across dates) for nocturnally biased drifting in the largest mayfly nymphs.

Behavioural observations

We made behavioural observations on the alpine bullhead–mayfly interaction in the laboratory in Sep-

tember 1995. Fish used in the experiments (mean standard length, 72 mm; SD, 17; $n = 10$) were maintained in 20-L aquaria. They were fed with living stream invertebrates each day, but no food was offered during the last 24 h before the observations. Nymphs of *B. rhodani* were collected from a stream with a high density (*c.* 0.9 m^{-2}) of benthic fish (bullhead and stoneloach, *Barbatulus barbatulus* (L.)). Nymphs were sorted and size-matched in the laboratory, and acclimated to experimental conditions for 4 h before the observations. We used 15 relatively uniformly-sized (mean head width, 0.72 mm; SD, 0.17; $n = 30$) nymphs in each aquarium. This density (190 m^{-2}) is at the lower end of density of *Baetis* nymphs in our study streams. The observations were made in aquaria ($40 \times 20 \times 20$ cm) where a current was created by pumping air through a diffuser (for a detailed description of the aquarium, see Tikkanen *et al.*, 1994). Current velocity near the bottom was 15–20 cm s^{-1}, which was within the velocity range encountered by *Baetis* in the field. The aquaria were filled with oxygenated tap water, which was changed after each observation. The substratum consisted of light-coloured stones collected from a nearby fishless stream. To avoid depletion of periphyton, stones were replaced each day during the experiment. A new set of nymphs was used for each observational run, and each fish was used only once.

Foraging behaviour of bullhead and the bullhead–mayfly interaction were recorded on videotape using an infrared sensitive videocamera (Philips CDD, model LDH 0600/10, Paris, France). Light was provided by fluorescent lights during the day and by infrared light at night. Light cycle (12L:12D) and water temperature (daily mean of 8 °C) paralleled field conditions in late September. The behaviour of *Baetis* nymphs and of the fish was recorded for 2 h during both day and night. We first recorded the behaviour of *Baetis* nymphs for 1 h without any fish; then we added one bullhead to the aquarium and, after 5 min of acclimatization, we continued recording for another hour. To prevent any carry-over of fish chemicals, different aquaria (as well as new sets of *Baetis* nymphs and fish) were used for the day and the night observations. The aquaria were thoroughly cleaned between the days of the experiment.

From the videotapes, we calculated the proportion of time spent by fish moving actively. We also counted the number of drift entries by *Baetis* and categorized them as non-contact (initiated without physical contact with the predator), swim-contact (contact with the body or fins of a swimming fish, not associated with an attack) or attack-contact. Finally, we measured the reactive distance of prey (distance to fish at the point of drift entry). We used Student's *t*-test to examine differences between night and day regarding (1) sculpin activity (percentage of time spent actively foraging, arc–sin square-root transformed) and (2) the number of drift entries initiated after a fish contact (no transformation needed). Repeated measures ANOVA was used to detect differences, if any, in the drift rate (log-transformed) of *Baetis* nymphs before and after fish introduction at different times of day.

Results

Diel variation in drift probability

In July, the probability of *Baetis* nymphs entering the drift was higher by day than by night in both fishless streams. In streams with trout, drift of *Baetis* was concentrated into the short period of darkness around midnight. A similar, though weaker, trend for nocturnal activity was observed also in the sculpin stream (Table 1). In August, drift in the two fishless streams was diurnal or slightly nocturnal, while in all streams with fish drift probability was markedly higher at night than by day. In September, the drift probability in both fishless streams indicated aperiodic drifting, whereas nocturnal drift probability greatly exceeded that by day in both streams with trout and, to a lesser extent, in the sculpin stream (Table 1).

Dipteran larvae did not show much diel variation in their drift probability. The only indication of nocturnal drifting was in September in the streams containing sculpin. In August, in the trout stream, drift of dipteran larvae was slightly diurnal (Table 2a). In contrast, drift probabilities of trichopteran, plecopteran and ephemeropteran larvae ('other prey') showed the expected pattern: thus, drift in the fishless streams was, with one exception (fishless stream 2 in August), aperiodic or diurnal (in July), while drift probability in streams containing trout was much higher by night than by day (Table 2b). Drift of these potential prey was also slightly nocturnal in the stream where sculpin was the only fish species.

Diel size distribution of drifting Baetis nymphs

The tendency for *Baetis* nymphs to become nocturnal as they grow larger was clear in the stream containing both trout and bullhead, whereas in the stream with only trout, this was evident only in August (Fig. 1). In the bullhead stream, large *Baetis* nymphs drifted nocturnally in August, but not in July, whereas the intermediate-sized nymphs always drifted nocturnally (the largest size class was missing in September). Small nymphs were arhythmic drifters in all fish streams. Drift of all size classes was aperiodic in both fishless streams, although there was a non-significant tendency for the largest nymphs to become nocturnal in August and September in these streams also (Figs. 1b & c). Overall, the tendency for the two largest size classes of *Baetis* (head width > 0.45 mm) to drift mainly at night was highly sig-

nificant in all fish streams (Fisher's combined probability test, trout stream: χ^2, $P < 0.001$; bullhead stream: $P < 0.001$; trout + bullhead stream: $P < 0.001$). No such trend was observed in the two fishless streams.

Laboratory observations of bullhead−mayfly interactions

Bullheads were almost exclusively night-active (*t*-test for diel differences in activity: $t = 6.85$; 4 d.f.; $P = 0.006$). In darkness, they moved around the aquarium with short bouts of swimming interrupted by relatively long inactive periods. After detecting a mayfly nymph, a fish typically would stop, orientate towards the prey and stay motionless for a few seconds ('listening' for prey with its lateral line; Janssen, Coombs

Table 1 Drift probability ($\times 10^{-5}$) of *Baetis* mayfly nymphs. Underlined and bold numbers indicate a two-fold or five-fold greater probability of drift by night than by day, respectively. For the exact method of calculation, see text

Stream type	July			August			September		
	Night	Day	*n*	Night	Day	*n*	Night	Day	*n*
Fishless stream 1	490	752	547	396	1756	1056	737	496	790
Fishless stream 2	709	1616	1101	1373	1048	950	705	783	4690
Sculpin	<u>427</u>	<u>121</u>	479	<u>2431</u>	<u>953</u>	491	1521	914	550
Trout	**1010**	**186**	58	<u>528</u>	<u>196</u>	155	<u>427</u>	<u>121</u>	255
Trout + sculpin	<u>470</u>	<u>180</u>	554	**1385**	**168**	145	**2092**	**243**	954

Table 2 Drift probability ($\times 10^{-5}$) of (a) dipteran larvae and (b) other prey types. Underlined and bold numbers indicate a two-fold or five-fold greater probability of drift by night than by day, respectively. For the exact method of calculation, see text. Dipteran larvae consist of blackfly (Simuliidae) and midge larvae; other prey types comprise stonefly and mayfly (other than *Baetis*), nymphs and caddis larvae

| Stream type	July			August			September		
	Night	Day	*n*	Night	Day	*n*	Night	Day	*n*
(a) Dipteran prey									
Fishless stream 1	457	828	496	675	718	283	1116	1907	133
Fishless stream 2	36	74	408	79	63	133	126	129	1070
Sculpin	604	710	250	38	20	841	<u>565</u>	<u>142</u>	407
Trout	200	160	152	385	1124	105	576	671	43
Trout + sculpin	1430	942	114	1061	566	200	<u>118</u>	<u>30</u>	86
(b) Other prey									
Fishless stream 1	69	299	75	194	138	64	899	1311	109
Fishless stream 2	31	693	468	<u>1413</u>	<u>354</u>	49	44	97	772
Sculpin	77	57	52	<u>70</u>	<u>24</u>	188	23	20	87
Trout	1427	894	131	<u>8</u>	<u>3</u>	27	**1618**	**42**	22
Trout + sculpin	<u>1720</u>	<u>533</u>	24	**1659**	**195**	38	<u>113</u>	<u>30</u>	62
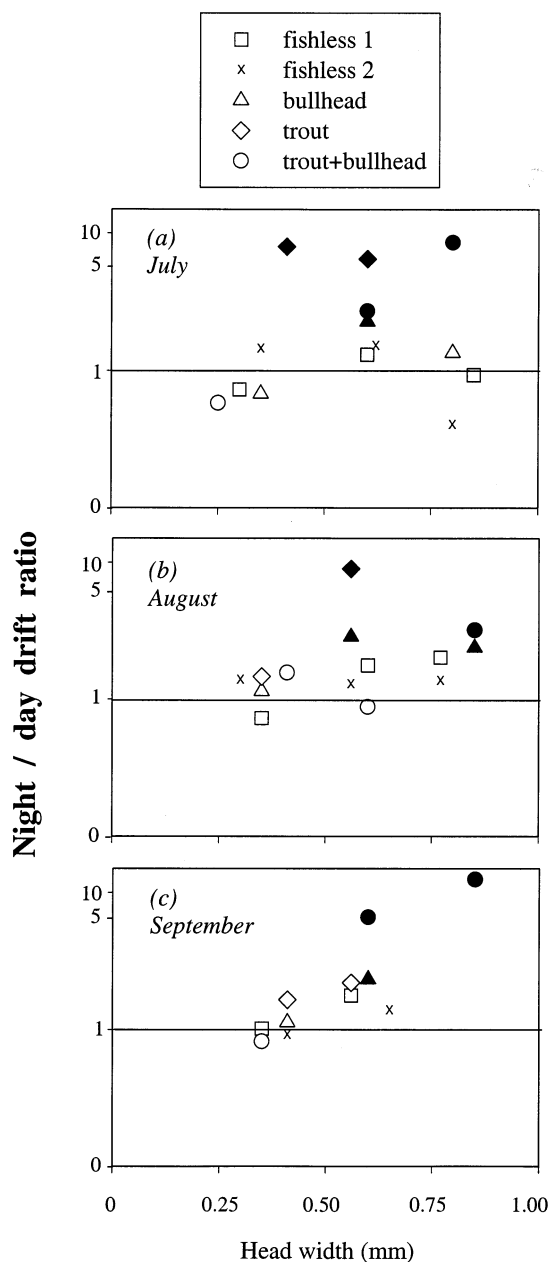
Fig. 1 Night versus day ratio of *Baetis* drift densities (n m^{-3}) as a function of *Baetis* size in (a) July, (b) August and (c) September. Mayfly nymphs were grouped into three size classes based on their head widths (< 0.45 mm, 0.45–0.75 mm and > 0.75 mm). The exact position of each symbol along the x-axis indicates the average head width of *Baetis* nymphs in each size class. On some occasions, only two size classes were present in a stream. A filled symbol indicates a statistically significant deviation (as shown by the binomial test with Bonferroni correction) from the night/day ratio of 1.0.

& Pride, 1990) before launching a strike. Bullheads stayed in close contact with the substratum, and they

never attacked drifting prey. Overall, the proportion of successful attacks by bullheads on *Baetis* nymphs was 28%.

Drift of *Baetis* nymphs was clearly nocturnal (repeated measures ANOVA, main effect of time: $F = 105.1$; 1 d.f.; $P < 0.001$), and it increased significantly during both day and night after bullhead introduction (main effect of fish: $F = 17.2$; 1 d.f.; $P = 0.003$) (Fig. 2a). The interaction term was also significant (fish × time: $F_{1,8} = 6.0$; $P = 0.04$), indicating that drift was more distinctly nocturnal in the presence than in the absence of bullheads. Most drift entries (70–90%) were initiated without physical contact with fish, but the frequency of fish-induced drifting was significantly higher at night than by day (Fig. 2b; $t = 3.65$; 4 d.f.; $P = 0.032$). Drift as an active escape from bull-
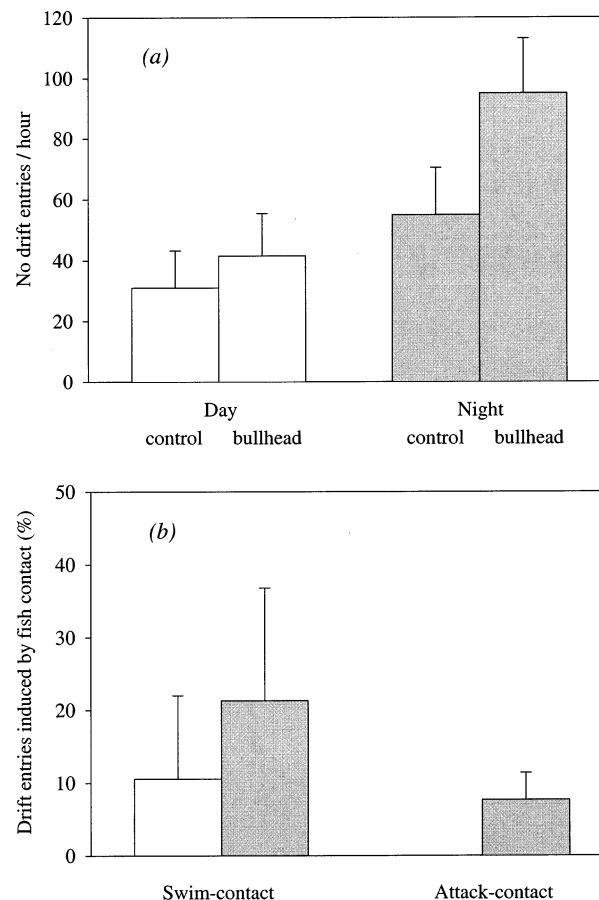

Fig. 2 Drift of *Baetis* nymphs in the laboratory trials. (a) Number of drift entries per hour in control and fish aquaria during the day and the night. (b) Proportion of drift entries initiated after a swim or attack contact with alpine bullhead in the day (open bars) and the night (filled bars) trials. Bars indicate means ± SE, $n = 5$.

head attack occurred only in darkness, whereas all drift entries induced by fish in daylight were caused by swim-contacts not associated with an attack. Swim-contacts also caused many nymphs to start drifting at night. The average drift initiation distance was 16.6 cm (SD, 10.0; $n = 60$) for non-contact, 3.3 cm (SD, 1.2; $n = 56$) for swim-contact, and 2.9 cm (SD, 1.1; $n = 32$) for attack-contact drift entries (one-way ANOVA for differences between the categories: $F = 67.4$; $P < 0.001$).

Discussion

Mechanistic explanations for the nocturnal drift of lotic invertebrates have centred around the predator avoidance hypothesis of Allan (1978). The fact that the largest prey, in particular, postpone drifting until darkness has now been documented experimentally in many laboratory studies (McIntosh & Townsend, 1994; Tikkanen *et al.*, 1994) and at least one field study (Douglas *et al.*, 1994). It has also been shown that fish chemicals *per se* may trigger the avoidance response in some mayfly nymphs (Douglas *et al.*, 1994; Tikkanen *et al.*, 1994), although the response is stronger when prey are subjected to a variety of stimuli simultaneously (Scrimgeour, Culp & Cash, 1994; Tikkanen, Muotka & Huhta, 1996). Many studies have demonstrated that the foraging activity of both invertebrate (Malmqvist & Sjöström, 1987; Lancaster, 1990; Peckarsky, 1996) and vertebrate (Culp *et al.*, 1991; McIntosh & Townsend, 1994; Scrimgeour & Culp, 1994; Tikkanen *et al.*, 1994) predators may increase drift in stream invertebrates. In a laboratory experiment, McIntosh & Townsend (1994) showed that a nocturnal increase in the drift of a siphlonuriid mayfly resulted from a higher frequency of encounters with fish (the common river galaxias) at night compared to day. Galaxids are mainly nocturnal foragers that use mechanical cues to detect prey. Using this foraging mode, they disturb mayflies from the substratum, thus raising drift rates in darkness. Similarly, Peckarsky (1996) reported an enhancement of nocturnal drift by *Baetis* nymphs in response to encounters with the predacious stonefly (*Megarcys signata* (Hagan)). In our experiments, bull-head induced nocturnal drifting of *Baetis* mayflies both as a result of passive prey disturbance (from swim-contact) and active escape following fish attacks.

It should always be in the interest of the prey to interrupt a predation cycle at the earliest possible stage

(Endler, 1991). By constraining drift to darkness, lotic mayflies may completely avoid encounters with visually hunting fish. However, the nocturnal activity of mayfly nymphs seems to risk contact with nocturnally active, benthic fish that use hydrodynamic cues for prey detection. These predators may be evaded after a close contact, or even an attack, by entering the water column and drifting downstream.

The original avoidance hypothesis to explain drift periodicity predicts that stream invertebrates depress daytime drift in response to the presence of drift-feeding fish (Allan, 1978; Douglas *et al.*, 1994). This was well supported by our diel drift samples from the trout streams compared with the fishless streams. In contrast, drift of macroinvertebrates in the bullhead stream by day was not suppressed by the presence of bullhead. Apparently, entering the water column in daylight does not incur such a high risk in streams containing only benthic fish, and suppression of daytime drift would not be beneficial to the prey when only bottom-feeding fish are present. In streams with bottom-feeding fish, nocturnal drift peaks should thus be caused by an increased frequency of fish-invertebrate encounters at night rather than by reduced daytime activity. Thus, the reason for the diel drift pattern in streams with bottom-feeding fish should be opposite to the prediction of the classical avoidance hypothesis.

Another central prediction of the avoidance hypothesis is that the prey types most vulnerable to predation should show the strongest response. This prediction was well born out by our data. First, dipteran larvae, which are often underrepresented in fish diets (e.g. Sagar & Glova, 1995), were relatively non-responsive to the presence of fish. Further, the strongest bias towards nocturnal activity was found, as expected, in large *Baetis*. Interestingly, mayflies in our experiments drifted nocturnally even in the absence of cues from fish, suggesting that drift periodicity may include a fixed component. Recent studies have shown that the response of mayflies to fish chemicals is asymmetrical; nymphs from trout streams maintain nocturnal activity even when fish cues are removed, whereas nymphs from fishless streams respond flexibly to fish manipulation (Cowan & Peckarsky, 1994; McIntosh & Townsend, 1994, 1995). Our study provides some evidence that mayflies from streams with benthic fish also tend to drift nocturnally in the absence of any fish cues. It may well be that this tendency for nocturnal

activity is a generalized response to fish chemicals, and this behavioural trait is more or less inflexible. The proximity of an actively foraging fish would then allow a more accurate assessment of risk, leading to stronger antipredator responses when the prey are exposed to multiple fish cues (see Scrimgeour *et al.*, 1994; Tikkanen *et al.*, 1996).

Given that the nocturnal drift peak may be caused at least in part by night-active bottom-feeding fish, an intriguing question is whether predatory invertebrates could also modify the drift periodicity of their prey. The activity periods of predatory stonefly nymphs often overlap with those of their mayfly prey, i.e. many stoneflies are nocturnal or crepuscular foragers (Sjöström, 1985; Peckarsky & Cowan 1995; Huhta, Muotka, Juntunen & Yrjönen, 1999). Upon close contact with a foraging stonefly, *Baetis* nymphs typically enter the water column, followed by a rapid burst of swimming (Peckarsky, 1996; Tikkanen, Muotka, Huhta & Juntunen, 1997). Therefore, it is not surprising that Malmqvist & Sjöström (1987) observed a nocturnal increase in the drift of *B. rhodani* nymphs after the density of a predaceous stonefly nymph (*Dinocras cephalotes* (L.)) was experimentally doubled in a section of a Swedish stream. Our field observations, however, suggest a minimal role for invertebrate predators in regulating drift periodicity: drift in the two fishless streams was aperiodic or slightly diurnal, although the density of invertebrate predators in these streams was among the highest of those in our study streams. Furthermore, in a series of laboratory trials, we have shown that the presence of a perlodid stonefly nymph (*Diura bicaudata* (L.)) has only subtle, if any, effects on the drift periodicity of *B. rhodani* nymphs (Huhta *et al.*, 1999). It thus appears that predatory invertebrates may contribute to nocturnally biased drifting of stream invertebrates, but only if their density is unusually high.

Our evidence on the potential role of benthic fish in inducing the nocturnal peak of macroinvertebrate drift rests on nonexperimental, comparative data. The major problem when such data are used for testing hypotheses is that different areas may vary in relation to many other factors, in addition to the one the investigator is interested in, and replication is small. Manipulative experiments certainly provide the strongest tests, but they are not always feasible, especially at scales relevant to the processes studied. We fully concur with Alatalo, Gustafsson & Lundberg (1986) that evidence from natural experiments should not be ignored if reasonable alternative explanations cannot be pointed out. In our study, an obvious alternative explanation is that the presence of invertebrate predators might swamp the effects of fish on diel drift patterns. However, this mechanism is not likely to produce the diel differences observed among our study streams (see above), and we therefore conclude that our study provides reasonable evidence for the role of bottom-feeding fish in inducing nocturnally biased drifting of lotic invertebrates. It must be stressed, however, that the lack of replication in our study leads to a relatively weak test of the hypothesis, and large-scale experimental manipulations of the fish assemblage composition are needed to provide a more conclusive test.

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