Behavioural interactions in stream food webs: the case of drift-feeding fish, predatory invertebrates and grazing mayflies

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Summary

1. We examined direct and indirect behavioural responses of grazing mayfly nymphs (*Baetis rhodani*) to predation risk from a drift-feeding fish (European minnow; odour manipulated), and two types of invertebrate predators, *Diura bicaudata* (stonefly) and *Rhyacophila nubila* (caddis larva). We also assessed the direct responses of the invertebrate predators to fish chemicals.

2. Based on diel gut content periodicity, *D. bicaudata* nymphs were strongly nocturnal foragers. *R. nubila* was also nocturnal, but only in a fish stream; in a stream lacking fish, their gut contents did not vary significantly on a diel basis. In the laboratory, *Diura* was nocturnal even in the absence of fish and almost ceased moving when fish was present. *Rhyacophila* shifted from aperiodic to nocturnal foraging in the presence of fish.

3. The contrasting behaviours of the two predators may be explained by their respective field distributions: *D. bicaudata* always co-occurs with fish, whereas *R. nubila* is also found in streams without fish. Therefore, a capacity for plastic anti-fish responses is beneficial for *R. nubila*, but not for *D. bicaudata*.

4. Drift of large *Baetis* nymphs was aperiodic when *Rhyacophila* was present, but nocturnal in all other treatments. Drift rate was highest when both *Diura* and fish were present and lowest in treatments with *Rhyacophila*. Predatory stoneflies, but not the caddis larva, induced a night-time peak in the drift of both *Baetis* size-classes. In the absence of predators, small *Baetis* drifted aperiodically. Interaction terms in three-way ANOVAs testing for the indirect effects of fish and invertebrate predators on mayfly drift periodicity and drift rate were all non-significant, indicating that the response of *Baetis* to one type of predator was not modified by the other predator.

5. Our results indicate that the drift periodicity of lotic mayflies may be fine-tuned to variations in the multi-predator environment and that prey responses are size-specific. We conclude that the effects of invertebrate predators on prey behaviour vary in relation to predator's foraging strategy and generalizations based on studies with only one type of predator should be avoided.

Key-words: anti-predator behaviour, diel periodicity, multi-predator environments, predator–prey interactions, stream drift.

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Introduction

Virtually all prey animals live in environments where they are confronted with many types of predators simultaneously. It is thus somewhat surprising that a vast majority of research on anti-predator

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behaviours deals with one predator–one prey situations, notwithstanding their apparent unrealism. Multiple predation risk places the prey in a conflicting situation where they must balance relative risks from different types of predators, and avoidance of one predator often increases exposure to others. For example, small prey fish moving into shelters avoid encounters with piscivorous fish, but are simultaneously exposed to predatory crayfish that forage in crevices. Crayfish, in turn, may force the prey to abandon shelters, thus enhancing their vulnerability to larger fish (Rahel & Stein 1988). Similarly, Resetarits (1991) showed that avoidance of either a predatory salamander or brook trout increased the exposure of a crayfish prey to the other predator. However, the combined effect of these predators on the growth and activity of the crayfish were non-additive, probably because the indirect, positive effects of brook trout partly compensated for their direct, negative effects.

Such predator–predator interactions may have far-reaching effects on prey assemblage structure, as demonstrated by Kotler, Blaustein & Braun (1992) for desert rodents and by Leipold & Tessier (1991) for competing *Daphnia* species. Differential vulnerability of consumers to different types of predators may even elicit trophic cascades in seemingly complex food webs characterized by a high degree of omnivory (Power, Marks & Parker 1992). Thus, indirect effects associated with multi-predator situations are clearly important in many natural systems and, as pointed out by Huang & Sih (1991), a mechanistic understanding of such effects necessitates that behavioural interactions between constituent species be quantified.

Stream organisms live in an environment where they face multiple predation risks from both invertebrate (e.g. stonefly and caddisfly larvae) and vertebrate (e.g. fish) predators. Yet most studies on the anti-predator behaviours of lotic invertebrates have addressed only one of these predator types. For example, it is well known that stream invertebrates enter the water column to drift downstream mainly at night to avoid encounters with day-active, drift-feeding fish (Cowan & Peckarsky 1994; Douglas, Forrester & Cooper 1994; McIntosh & Townsend 1994; Tikkanen, Muotka & Huhta 1994). Similarly, invertebrate predators often evoke increased drift rates in mayfly nymphs (Peckarsky 1980; Malmqvist & Sjöström 1987; Lancaster 1990; Kratz 1996; Tikkanen *et al.* 1997), although their role in inducing drift periodicity is less well studied. In a series of laboratory experiments, Soluk & Collins (1988a,b) and Soluk (1993) showed that the numerical impacts of fish (sculpin) and stonefly predators on mayfly prey are not simply additive, but are instead modified by behavioural interactions between the preda-

tors and prey, as well as between different types of predators.

In this study, we examined both direct and indirect behavioural responses of grazing mayfly nymphs to predation risk from a drift-feeding fish and two types of invertebrate predators. We also assessed the direct responses of the intermediate predators to fish presence. Our specific questions were: (i) Do invertebrate predators modify their diel activity patterns in response to fish chemicals? (ii) Do invertebrate predators induce nocturnal peaks in the drift of lotic mayflies, and do the responses differ when prey are subjected to mobile (stonefly nymphs) vs. relatively sedentary (caddis larvae) predators? (iii) Do mayflies express predator-specific drift responses, and does the combined risk of fish and invertebrate predators modify prey responses from those expressed to each predator type separately?

Materials and methods

STUDY ANIMALS

The species constituting our study system, the grazing mayfly nymph *Baetis rhodani* Pict., the predatory invertebrates *Diura bicaudata* (L.) (Plecoptera) and *Rhyacophila nubila* Zett. (Trichoptera), and European minnow (*Phoxinus phoxinus* L.), a drift-feeding fish, are all conspicuous members of the stream fauna in northern Finland. *Baetis* nymphs feed primarily on periphytic algae on stone tops, and they commonly enter the water column and drift downstream, especially in darkness (e.g. Kohler 1985). *D. bicaudata*, a predatory stonefly nymph, occupies lake outlets and stony lake shores (Brinck 1952). It prefers semi-sessile invertebrates, such as simuliid and chironomid larvae in its diet (Tikkanen *et al.* 1997). *Diura* stoneflies are mobile predators that hunt within the interstitial spaces among stream stones and, like most perlodid stoneflies (Allan, Flecker & McClintock 1986), are reluctant to enter stone tops. *R. nubila* is a freely living predatory caddis larva, which feeds disproportionately on sedentary prey types (Muotka 1993). While hunting on blackfly larvae, *Rhyacophila* are able to manoeuvre in high-velocity microhabitats on tops of stones (Malmqvist & Sackmann 1996), but when only mobile prey types are available, they turn into ambush hunting, feeding on any benthic prey that fall within their reach (Otto 1993). Both these predators readily consume *Baetis* nymphs, although these rarely form the bulk of the diet of either one of the species (Muotka 1993; Tikkanen *et al.* 1997). The top predator in our system, the European minnow, hunts in daylight on prey drifting in the water column (Müller 1973). Minnows are reported to be nocturnally active in winter (Greenwood & Metcalfe 1998), and Huusko & Sutela (1997) observed a substantial amount of nocturnal activity in late spring

to early summer at water temperatures ranging from + 5 to + 10°C. The exact temperature where minnows exhibit the autumnal shift to nocturnal (or aperiodic) activity is not known, but in our earlier experiments (see Tikkanen *et al.* 1994) we have found minnows to be active throughout the day in late September; however, in these experiments, they were able to capture drifting prey only in daylight. We thus conclude that at the time of our experiments minnows posed the greatest threat to drifting mayflies during the day. *Baetis* is commonly a major dietary item for minnow, and they also readily attack and consume predatory invertebrates (A. Huhta, personal observation).

Most of the animals used in our experiments were collected from the outlet of lake Välilampi (66°14′N, 29°10′E); however, additional collections of minnows were made from other streams in the same river system. This outlet stream supports a diverse fish community including both drift-feeding (minnow and brown trout, *Salmo trutta* L.) and benthic fish (eastern sculpin, *Cottus poecilopus* Heckel and burbot, *Lota lota* (L.), but with a relatively low overall density (≈ 0.20 fish m^{-2}).

GUT CONTENT PERIODICITY AND GUT EVACUATION RATES OF *DIURA* AND *RHYACOPHILA*

We collected 20–25 *R. nubila* larvae at 4-h intervals over a 24-h period in September 1996 from two streams, one containing no fish, and one supporting dense populations of brown trout and eastern sculpin (combined density: 1.3 fish m^{-2}). Similar collections were also made for *D. bicaudata* nymphs, but only from the fish stream, because this species does not occur in the few streams without fish found in our study area. Upon collection, the animals were quickly deep-frozen and later transferred to 70% ethanol. Within a few weeks, they were processed for the gut contents analysis: their head widths were measured and the foregut contents weighed to the nearest microgram after drying at 60°C for 24 h. Head widths were converted to dry masses using regressions developed for each species. Care was taken to ensure that field-collected specimens were within the same size range as those used in the laboratory experiments. Early fifth instar *Rhyacophila* larvae were collected to ensure that individuals from different streams were of the same size. Mean head width (± 1 SD) of fish stream rhyacophilids was 1.00 ± 0.21 mm, compared to 0.96 ± 0.19 mm for larvae found in streams without fish ($n = 40$ for both).

The null hypothesis of no diel differences in the gut fullness of live-collected specimens was tested with one-way ANOVA, followed by Tukey's tests for multiple comparisons. Before entering the analysis,

data were logarithmically ($\log x + 1$) transformed to reduce heterogeneity of variances.

When gut fullness is used as an indicator of feeding periodicity, gut evacuation rate should be closely coincident with the sampling interval used. We therefore measured the gut evacuation times of *Diura* and *Rhyacophila* in recirculating stream tanks. We collected 30–35 larvae of each species at the time of day when their guts were most full, as indicated by the diel gut content study. The animals were then transferred to stream tanks where no prey items were available. We placed a few sterile stones (animals and periphyton removed) in each tank to provide the animals with suitable sheltering places. Six individuals of both species were preserved immediately upon collection, and thereafter six to eight individuals were collected at intervals of 2, 4, 8 and 12 h. All animals were first deep frozen, then transferred to 70% ethanol and their gut contents were later processed as described above for the diel gut content study. Water temperature during the experiment ranged between 4 and 7°C.

BEHAVIOURAL EXPERIMENTS

In these experiments, we tested (i) the influence of vertebrate and invertebrate predators on the drift behaviour of mayfly larvae, and simultaneously, (ii) the influence of fish on the diel periodicity of the invertebrate predators. The experiments were conducted at Oulanka Biological Station, northern Finland, between 12 and 15 September 1995 (experiments with *Diura*), and between 11 and 14 September 1996 (with *Rhyacophila*). We used recirculating stream tanks ($40 \times 20 \times 19$ cm), where the flow is created with air pumped through a diffuser. Current velocity near the experimental arena at the bottom of the aquarium is ≈ 15 cm s^{-1}, which is well within the range of velocities where all these species occur in nature (for a more detailed description of the stream system, see Tikkanen *et al.* 1994). A dense mesh cloth was fastened to the bottom of the arena to provide a foothold for the animals. The experimental substratum consisted of eight unglazed ceramic tiles ($5 \times 5 \times 0.5$ cm) arranged in two rows parallel to the flow. The tiles were raised from one edge by a shallow support so that the animals had unconstrained access to the undersides of stones. This microhabitat was used extensively by both *Baetis*, *Rhyacophila* and *Diura*, so it did not constitute a refuge from invertebrate predation. To provide food (periphytic algae) for mayflies during the trials, the tiles were incubated in a nearby stream without fish for 2–3 weeks. Our previous studies using the same stream system and a similar set-up have shown that *Baetis* nymphs do not deplete algal resources during short-term (less than 24 h) trials (Tikkanen *et al.* 1994; Tikkanen, Muotka & Huhta

1996). To ensure that no fish chemicals were unintentionally introduced into the study system, water for the experiments was transferred from a stream without fish. Water temperature, which was monitored continuously during the trials, paralleled natural stream temperatures, ranging from 5 to 9°C. Light was provided by fluorescent lights during the day and observations in the dark were facilitated by a dim red light, which had no visible effects on the behaviour of the invertebrates (see also Culp & Scrimgeour 1993; Peckarsky & Cowan 1995). Lights were turned off 1 h before the night observations began.

Our experimental design was a repeated measures, two-factorial ANOVA with nine replicates for each treatment combination. The experimental factors were (1) predation risk (a between-subject factor with four levels: control, invertebrate predator present, invertebrate predator + fish odour present, mere fish odour present); and (2) time of day (a within-subject factor with two levels: day vs. night). Fish odour was used instead of a live fish due to logistical reasons (ease of operation and observation of invertebrate behaviour), and because previous experiments have shown that mere fish chemicals are needed to trigger avoidance responses in lotic mayflies (Cowan & Peckarsky 1994; Douglas *et al.* 1994; Tikkanen *et al.* 1994). Each stream tank received 20 mayflies, 10 small (mean body length, excluding cerci ± 1 SD $= 2\cdot71 \pm 0\cdot59$ mm, $n = 30$) and 10 large ($5\cdot16 \pm 0\cdot58$ mm; $n = 30$) nymphs. This density of *Baetis* (256 nymphs m^{-2}) is within the range of densities observed in the field in late autumn. The same set of nymphs was used in all trials during a day. Treatments with invertebrate predators received two specimens of either *Diura* (mean body length, cerci excluded ± 1 SD $= 8\cdot69 \pm 1\cdot7$ mm, $n = 20$) or *Rhyacophila* ($11\cdot8 \pm 3\cdot6$ mm, $n = 28$). This density ($38\cdot5$ ind m^{-2}) is also well within the natural densities of both these species in our study streams. To standardize the hunger level of the predators, we kept them without food in circular holding tanks (see Mackay 1981) for 24 h before using them in the trials. Each predator was used only once (i.e. during 1 day's trials), after which they were measured and released back to the stream. Minnows were collected from a nearby stream using a back-pack type electrofishing gear. The fish were maintained for about 1 week before the trials in large (40 L) rearing tanks, where they were fed daily with live mayfly nymphs. After the trials, fish were measured and released to the stream. Fish odour was provided by keeping a live minnow in an oval plastic tube (diameter 5 cm) with netting at both ends. The tube was placed parallel to the flow on the horizontal baffle of the aquarium (see Tikkanen *et al.* 1994), so that invertebrates on the experimental arena could not see the fish, but water flowing through the tube carried fish chemicals. To control for any hydrodynamic effects of the tube, we added an empty tube to all other treatments.

The water and tiles were changed every morning and, to prevent carry-over of fish chemicals, the tanks were thoroughly cleaned with diluted detergent and hot water between the days of the experiment. We ran six trials a day and the treatments were randomly assigned to each of six aquaria. Invertebrate predators were introduced to the experimental arenas 16 h before the start of the trials, since our earlier experience with both *Diura* and *Rhyacophila* has shown that unless allowed an adequate acclimation period, they will behave more or less erratically. *Baetis* nymphs were collected from the field, sorted in the laboratory and placed in the stream tanks 2 h before the start of the trials. After a day's trials, mayflies were collected, counted and some of them were preserved for later size measurements. No mayflies were consumed by the predators during the trials. Tubes containing one minnow or no fish were also added 2 h before the first day observations began. We made behavioural observations four times a day: at 14.00–15.30 hours and 17.00–18.30 hours (day trials), and at 19.00–20.30 hours and 22.00–23.30 hours (night trials). The order in which the tanks were observed was randomized within each observation period.

During 15 min of observations per aquarium, we counted all drift entries (defined as entry into the water column) by differently sized *Baetis* nymphs, and categorized them as contact or non-contact drifts. A contact drift was initiated within an antennal distance of the invertebrate predator, but was not necessarily associated with an attack.

To study the activity periodicity of the invertebrate predators in the presence vs. absence of fish chemicals, we recorded the time spent moving by the invertebrate predators in each trial. In the beginning of each observation period, one of the predators was randomly selected as a focal individual, whose behaviour was monitored continuously for 15 min. We only recorded movements of one body length or longer (called major moves by Soluk & Collins 1988c), since these are probably closely associated with the foraging activities of the predators. Since there was considerable among-individual variation in the activity of the invertebrate predators, we conducted additional trials where the behaviour of the mayflies was not monitored. Thus, the number of replicates was 15 for both *Diura* and *Rhyacophila*.

Repeated measures one-way ANOVA was used to test for the effects of fish chemicals on the diel activity periodicity of the invertebrate predators (percentage of time spent moving, arc-sin square-root transformed). The null hypothesis of no differences in drift rates of *Baetis* among treatment combinations was tested with a repeated measures ANOVA,

followed by Tukey's test for pairwise comparisons. If needed, data were log $(x + 1)$ transformed to satisfy the homogeneity of variances assumption. The diel periodicity of mayfly drift was examined in more detail by calculating the mean night: day (N/D) drift density ratios for each predator treatment. Large values of N/D indicate nocturnal drifting, whereas 1·0 indicates aperiodic drifting. We used a binonomial test (Sokal & Rohlf 1981) to examine whether the observed N/D ratios deviated from unity; this was done separately for each predator treatment in both experiments. It must be emphasized, however, that these tests do not provide a direct test of behavioural modification among the different types of predators. For this purpose, we used a three-way rmANOVA with fish, invertebrate predator and time of day as factors, two levels for each factor. A significant *Diura/Rhyacophila**fish or *Diura/Rhyacophila**fish*time interaction would indicate an indirect effect, i.e. the effect of fish depends on the presence of the invertebrate predator, or vice versa (see, for example, Huang & Sih 1991).

Results

FIELD PERIODICITY OF THE INVERTEBRATE PREDATORS

The gut content periodicity of *D. bicaudata* nymphs indicated that they were strongly nocturnal foragers (Fig. 1; one-way ANOVA for diel differences in the relative dry weight of gut contents: $F = 2·92$, d.f. 5, 98, $P = 0·017$). Feeding of *R. nubila* was also nocturnally biased, especially in the fish stream ($F = 3·85$, d.f. 5, 98, $P = 0·003$; Fig. 2a). No significant diel variation occurred in the gut contents of larvae found in streams without fish ($F = 1·32$, d.f.
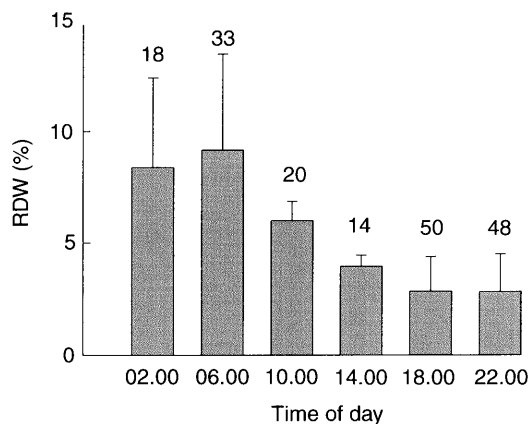

Fig. 2. Diel variation in the mean ($\pm$SE) RDW of *Rhyacophila nubila* nymphs from (a) a fish stream and (b) a stream without fish. Sampling intervals with a same letter do not differ significantly (Tukey's HSD test, $P > 0·05$). For other explanations, see Fig. 1.

5, 70, $P = 0·26$), although their gut mass also peaked during the night (Fig. 2b).

Gut clearance rates were very low for both species: there were no significant changes in gut contents during the 12-h experiment (1-way ANOVA, $P > 0·12$ for both species). Nonetheless, gut masses did decrease considerably during the first few hours of the experiment: in *D. bicaudata*, 65% of the initial weight of foregut contents remained after 4 h, and the corresponding figure for *R. nubila* was 53%. After that, gut evacuation ceased almost completely, with practically no further changes during the rest of the experiment.

DIEL FORAGING PERIODICITY OF THE INVERTEBRATE PREDATORS IN RELATION TO FISH PREDATION RISK

In the absence of fish, *D. bicaudata* was a distinctly nocturnal forager, whereas in the presence of fish, it strongly reduced its overall movement activity, both in daylight and in the dark (Fig. 3a). Thus, in rmANOVA, both main effects were significant (time

Fig. 1. Diel variation in the mean ($\pm$SE) relative dry weight of stomach contents (RDW, dry weight of stomach content as a percentage of predator dry weight) of *Diura bicaudata* nymphs. Figures above each bar indicate the proportion (%) of empty stomachs out of 20–25 guts examined per sampling interval.
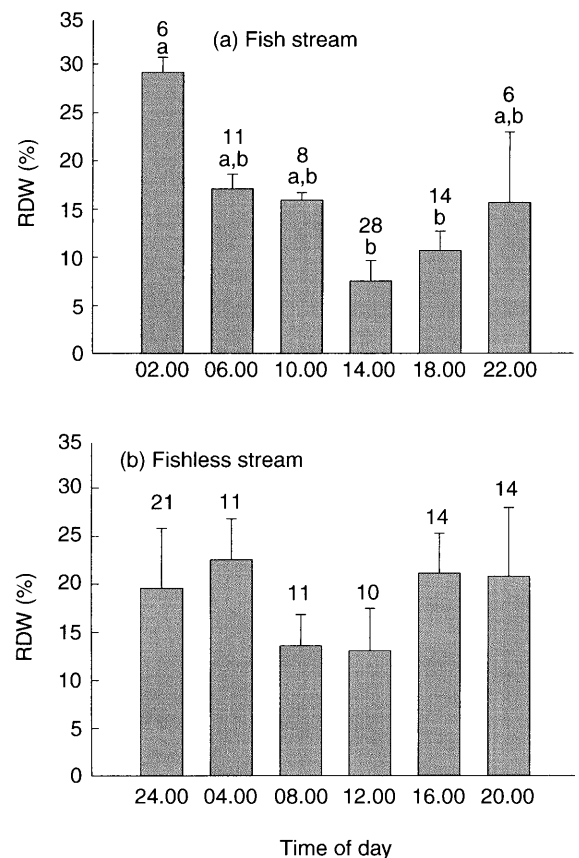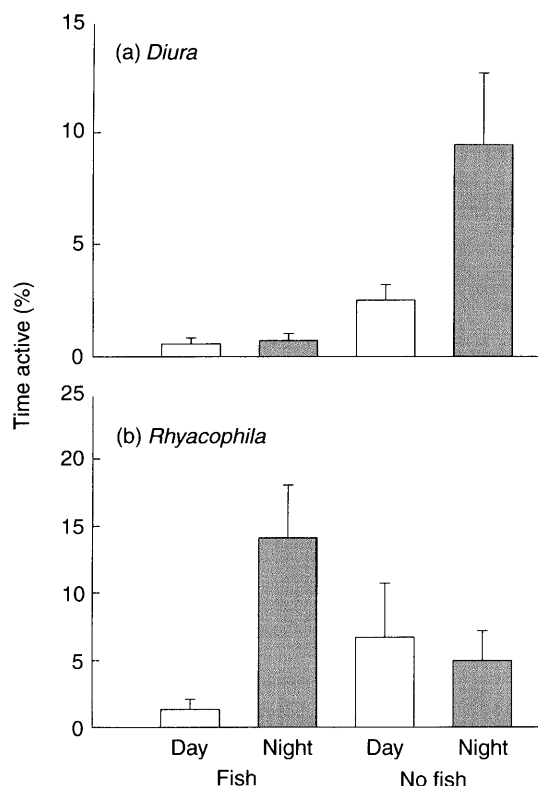
Fig. 3. Movement activity of (a) *Diura bicaudata* nymphs and (b) *Rhyacophila nubila* larvae at different times of the day, and in the presence vs. absence of fish chemicals. Vertical bars indicate 1 SE of the mean.

of day: $F = 5.94$, d.f. 1, 28, $P = 0.018$; fish presence: $F = 9.66$, d.f. 1, 28, $P = 0.003$), as was also the interaction term (time*fish: $F = 5.30$, d.f. 1, 28, $P = 0.025$).

The reaction of *R. nubila* larvae to fish presence was quite different from that of *Diura* (Fig. 3b). There was no overall change in movement activity (main effect of fish presence: $F = 0.41$, d.f. 1, 28, $P = 0.526$), but the larvae responded by altering their diel periodicity in the presence of fish: larvae released from fish cues were arrhythmic, whereas those exposed to fish chemicals turned strongly nocturnal (time*fish: $F = 5.20$, d.f. 1, 28, $P = 0.031$). Thus, the interaction term in repeated measures ANOVA was significant for both predators, but for different reasons. *Diura* was nocturnal in the absence of fish, but almost ceased moving when fish were present, regardless of the time of day, whereas *Rhyacophila* maintained its general level of activity and responded by shifting from aperiodic to nocturnal foraging in the presence of fish.

DRIFT OF *BAETIS* NYMPHS UNDER VARIABLE PREDATION RISK

Drift of large *Baetis* was nocturnal in the experiments where *Diura* was the invertebrate predator

(Fig. 4a; see also the significant main effect of time of day in rmANOVA, Table 1a). Nocturnality was most distinct when mayflies were subjected to *Diura* alone and least so in the controls, and in the treatments with both *Diura* and fish present. Drift rate was highest in the treatments containing both predator types, but the overall difference among the four predation risk levels was only marginally significant ($P = 0.077$; statistical power for detecting a significant difference was moderate, 0.56). Only 3% of all drift entries by large *Baetis* were directly caused by encounters with the stonefly. In the case of small *Baetis*, drift was nocturnal in all but the control treatments (Fig. 4b). Drift rates differed significantly between the predation risk levels (Table 1b), being highest in treatments involving fish cues, especially when *Diura* and fish were simultaneously present (significant pairwise differences shown by Tukey's test, $P < 0.05$: D + F > F > D = C). Proportion of drift entries directly induced by *Diura* was again very low, only 5% of all drifts.
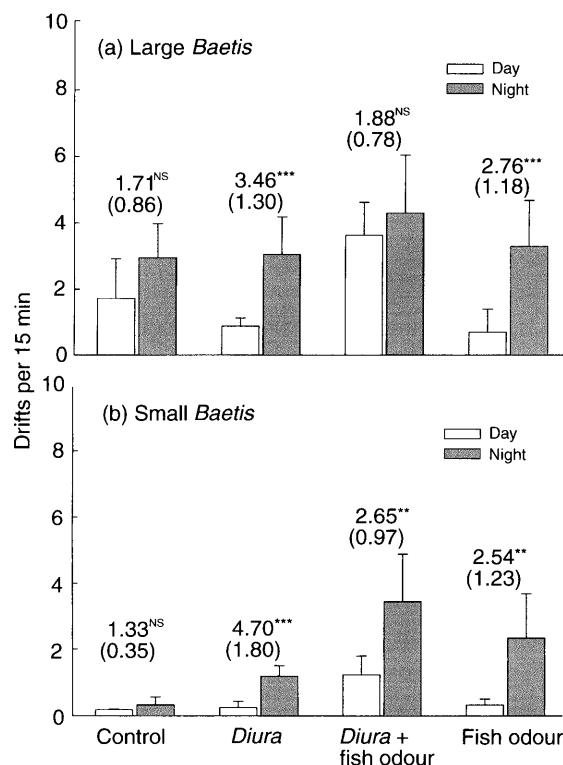

Fig. 4. Drift rates of (a) large and (b) small *Baetis rhodani* mayfly nymphs at different times of day, and in different predation risk treatments in the experiments using *Diura bicaudata* stonefly nymphs as the intermediate predator. Vertical bars indicate 1 SE of the mean. Figures above each bar are the mean ($\pm$ SE) night:day (N/D) drift density ratios in different predation risk treatments. Asterisk denote statistically significant tendency for nocturnal drifting (i.e. deviation from the N/D ratio of 1·0; binomial test,

Table 1. Repeated measures ANOVA for the effects of time of day (day vs. night) and predation risk (control vs. *Diura* vs. fish chemicals vs. *Diura* + fish chemicals) on the drift rates of large and small *Baetis* mayfly nymphs during 15-min trials

(a) Large *Baetis*

Source of variation	d.f.	MS	F	P
Within-subjects				
TIME	1	4·310	9·03	0·005
TIME*RISK	3	0·478	0·97	0·420
Error	32	0·480		
Between-subjects				
RISK	3	2·210	2·51	0·077
Error	32	0·880		

(b) Small *Baetis*

Source of variation	d.f.	MS	F	P
Within-subjects				
TIME	1	3·252	9·13	0·005
TIME*RISK	3	0·291	0·16	0·920
Error	32	0·373		
Between-subjects				
RISK	3	1·249	3·04	0·043
Error	32	0·410		

Table 2. Repeated measures ANOVA for the effects of time of day (day vs. night) and predation risk (control vs. *Rhyacophila* vs. fish chemicals vs. *Rhyacophila* + fish chemicals) on the drift rates of large and small *Baetis* mayfly nymphs during 15-min trials

(a) Large *Baetis*

Source of variation	d.f.	MS	F	P
Within-subjects				
TIME	1	21·67	2·92	0·097
TIME*RISK	3	11·46	1·54	0·221
Error	32	7·41		
Between-subjects				
RISK	3	16·24	2·31	0·094
Error	32	7·03		

(b) Small *Baetis*

Source of variation	d.f.	MS	F	P
Within-subjects				
TIME	1	0·008	0·02	0·892
TIME*RISK	1	0·353	0·83	0·481
Error	32	0·424		
Between-subjects				
RISK	3	0·223	0·78	0·520
Error	32	0·430		

In the experiments using *Rhyacophila* larvae as the intermediate predator, drift of large *Baetis* was nocturnal in both the control and fish odour treatments, but arrhythmic or even slightly diurnal in the *Rhyacophila* and *Rhyacophila* + fish treatments (Fig. 5a). Drift rates tended to be lower when *Rhyacophila* was present, but the main effect of predation risk in rmANOVA was only marginally significant (Table 2a; $P = 0·094$, power $= 0·53$). Small *Baetis* were aperiodic in all but the fish odour treatments, and the main effects of time and risk, as well as the interaction term, were all non-significant (Fig. 5b, Table 2b). The proportion of drifts directly induced by *Rhyacophila*–mayfly encounters was extremely low, $\approx 1\%$ for both *Baetis* size classes.

Finally, the interaction terms in rmANOVAs testing for the indirect effects of fish and invertebrate predators on mayfly drift rate and drift periodicity (e.g. *Diura**fish; *Diura**fish*time) were all non-significant (all $P > 0·14$), indicating that the response of *Baetis* to one type of predator was not modified by the presence of the other predator.

Discussion

Many studies have shown predatory stoneflies to be mainly nocturnal or crepuscular foragers (Allan 1982; Sjöström 1985; Peckarsky & Cowan 1995; Peckarsky 1996), but the effects of fish on their diel periodicity have been little studied. Nevertheless,
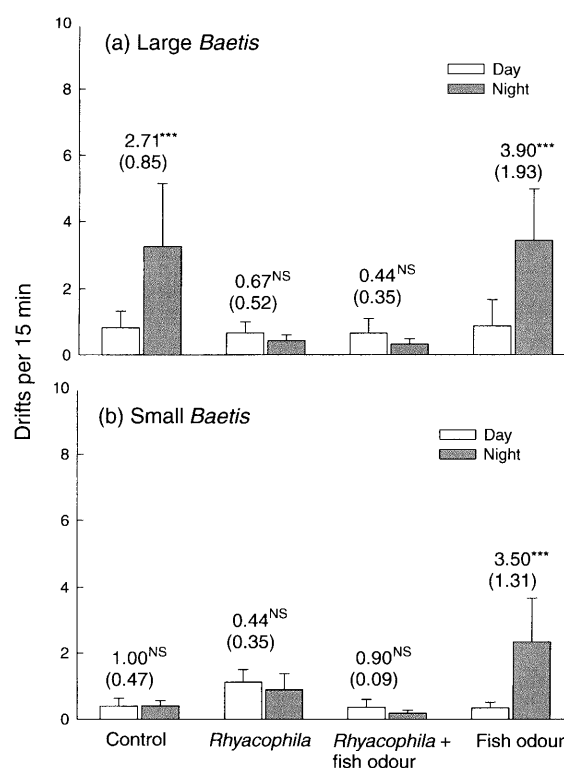

Fig. 5. Drift rates of (a) large and (b) small *Baetis rhodani* nymphs at different times of day and in different predation risk treatments in experiments using *Rhyacophila nubila* caddis larvae as the intermediate predator. For other explanations, see Fig. 4.

Soluk & Collins (1988c) observed a general reduction in the movement activity of *Agnetina capitata* larvae in the presence of sculpin. Moreover, this species was exclusively nocturnal, even in the absence of fish (see also Feltmate, Williams & Montgomorie 1992). Similarly, nymphs of *D. bicaudata* were nocturnally active in our experiments, but when exposed to fish chemicals, they almost ceased moving during both the day and the night. Obviously, such strong anti-predator responses must incur substantial costs and, indeed, it has been shown that perlodid stoneflies capture fewer prey in the presence vs. absence of fish (Soluk & Collins 1988a; Feltmate & Williams 1989; Scrimgeour & Culp 1994). This, in turn, leads to retarded growth and lower fecundity in nymphs exposed to fish predation compared to those in environments with no fish (Feltmate & Williams 1991).

The anti-predator responses of *Rhyacophila* larvae were quite different from those of *Diura*: the caddis was aperiodic in the absence of fish, but turned strongly nocturnal when exposed to fish chemicals. Thus, they responded flexibly to variations in fish predation regime. This capacity for flexible anti-predator responses was further substantiated by our field observations, which showed *Rhyacophila* to be night-active in a fish stream, but aperiodic in a stream lacking fish. The contrasting anti-predatory behaviours of the two species may be associated with their respective field distributions: *D. bicaudata*, being an inhabitant of stony lake shores and lake outlets, does not encounter fishless situations in the field, whereas *R. nubila* occurs in all kinds of running waters, including steep, heavily-shaded brooklets that rarely contain fish (A. Huhta, unpublished data). Therefore, it may have been beneficial for *R. nubila*, but not for *D. bicaudata*, to maintain a capacity for plastic anti-fish responses. *D. bicaudata* lives in an environment where fish predation risk is constantly high, and sampling the predation regime in such environments may incur a formidable mortality risk, selecting for inflexible predator avoidance behaviours (Sih 1987).

It is worth noting, however, that there was also a flexible component to the anti-predator behaviour of *D. bicaudata*: in the presence of fish, *Diura* reduced its general activity level to a minimum. Movement of a large invertebrate predator, such as *Diura* may generate hydrodynamic cues detectable by fish even in darkness. Fish presence may therefore force *Diura* to switch its foraging strategy from pursuing to an ambush-type of predation. This, however, does not necessarily incur a cost in terms of lost feeding opportunities, because when hunting on mobile prey types, such as *Baetis* nymphs, a sit-and-wait strategy may be at least as successful as a more active search mode (see Otto 1993). An interesting question is then whether *Diura* would reduce

its hunting activity in the presence of fish if more vulnerable, semi-sessile prey types (e.g. simuliid larvae; see Tikkanen *et al.* 1997) were available. These prey mainly occupy upper stone surfaces and hunting on them necessitates an active search mode, likely exposing the stonefly to fish. While visual predators are certainly impaired by darkness, an actively moving stonefly emits signals detectable by sculpins and other benthic fish using tactile or hydrodynamic cues for prey detection (Hoekstra & Janssen 1985).

Drift of large *Baetis* was nocturnal not only when exposed to fish or the stonefly predator, but also in control treatments devoid of any predator cues. It has recently been noted by many authors that fish stream mayflies remain nocturnal even in the absence of fish (Cowan & Peckarsky 1994; McIntosh & Townsend 1994), suggesting that nocturnally biased drifting may be a genetically fixed behavioural trait. Most previous experiments, however, have used only fish predators, and our results indicate that both drift periodicity and drift rates may be 'fine-tuned' to variations in the multi-predator environment. Thus, the presence of *Diura* alone enhanced the nocturnality of drift in large *Baetis* nymphs, but did not alter their drift rates. In contrast, simultaneous exposure to both *Diura* and fish not only induced higher drift rates in large *Baetis*, but also turned them more aperiodic drifters, due mainly to an increased frequency of daytime drifting. This may seem counter-intuitive: due to the risk posed by visually hunting fish, entering water column in daylight should be avoided by large prey (Allan 1978). It may be, however, that when exposed to many predator types simultaneously, mayflies perceive such a high risk that they tend to abandon risky patches immediately in search of less hazardous environments. In natural streams, fish are more or less patchily distributed, creating a mosaic of patches with variable fish densities (e.g. Muotka *et al.*, in press). In such environments, invertebrates may use drift not only as a means of locating unexploited food patches, but also patches of low predator density (see Forrester 1994; Tikkanen *et al.* 1994).

Small *Baetis* nymphs drifted aperiodically in controls with no fish and in treatments with *Rhyacophila*, but were nocturnal drifters in all other treatments. The presence of stonefly predators induced a night-time peak in the drift of small *Baetis*, an observation in concert with Malmqvist & Sjöström (1987) who found that when exposed to a perlid stonefly, small *Baetis* increased their nocturnal drifting far more than did the large nymphs. An opposite observation (large nymphs increased their drift rate in the presence of predatory stoneflies) was made by Corkum & Pointing (1979) and Lancaster (1990), who suggested that size-specific responses to

stonefly predators are caused by size-dependence in stonefly–mayfly encounter or attack rates. Our results, however, were clearly not caused by size-dependent interactions, because the proportion of drift entries directly induced by stoneflies was over-all very low and did not differ among *Baetis* size classes. Scrimgeour, Culp & Cash (1994) and Peckarsky & Penton (1989) also reported that most drift entries by *Baetis* occur without contact with the stonefly predator (or predator model), and Peckarsky (1996) suggested that alterations in may-fly drift rates in the presence of stoneflies represent both direct and indirect responses to predators. Nonetheless, stonefly predators display a hump-shaped preference curve, with strongest preference for intermediate-sized prey (Allan, Flecker & McClintock 1987). Large *Baetis* in our experiments were probably in a size refuge from predation by *Diura* of this size and, therefore, did not increase their drift rates in the presence of the stoneflies. Small *Baetis*, by contrast, were well within the size range commonly consumed by perlodid stoneflies of this size (Allan *et al*. 1987), and so probably experienced a substantially higher risk in the presence of an actively moving stonefly. Variable prey preference by stoneflies as a function of prey size may, indeed, explain much of the variation in size-dependent drift responses by mayflies. Despite large amounts of research on stonefly–mayfly interactions in streams, only a few studies have addressed size-dependent variation in encounter rates, capture successes and prey preferences, and the effects of these on the anti-predator behaviour of mayflies (but see Allan *et al*. 1987; Allan & Flecker 1988; Tikkanen *et al*. 1997).

While *Diura* seemed to exert relatively little control over the drift of mayflies, larvae of the caddis predator *Rhyacophila nubila* had a much stronger effect; when *Rhyacophila* was present, *Baetis* both reduced their general drift activity and turned aperiodic drifters, regardless of fish presence. Clearly, mayflies were able to recognize *Rhyacophila* as a potential threat, although no mayflies were killed during the trials. Whether the risk assessment was based on visual, hydrodynamic or chemical cues, cannot be determined based on our data, but Malmqvist (1992) observed no effect of *Rhyacophila* odour on the drift rate of *Baetis*. When only mobile prey types are available, *Rhyacophila* adopts an ambush hunting strategy (Otto 1993), and it may be that mayflies suppress their movement activity to reduce the likelihood of encounters with such a 'sit-and-wait' predator. This response (reduction of drift rates) was much stronger in large *Baetis*, suggesting that they are more at risk from predation by *Rhyacophila*. Unfortunately, we know of no studies documenting prey size selection by a rhyacophilid species. Simultaneous exposure to fish did not alter

the response, but when subjected to mere fish odour, both prey size-classes turned nocturnal.

In a recent review of studies on the drift responses of stream prey, Wooster & Sih (1995) showed that prey increased their drift rates in response to invertebrate predators more often than expected by chance, whereas effects of fish predators were more variable: some studies documented an increase, others a decrease in prey drift rate in the presence of fish. The authors suggested that this difference in prey emigration responses might explain why predatory invertebrates generally have a stronger impact on prey densities than do predatory vertebrates (see Wooster 1994). In our experiments, however, fish as well as stoneflies induced only subtle, if any, changes in the drift rates of *Baetis* nymphs, and the only significant response was the tendency of prey to drift less in the presence vs. absence of the caddis predator. This suggests that the effects of invertebrate predators on prey behaviour may depend on the type of the predator and that generalizations based on studies using only one type of predator, usually stonefly nymphs, may not be warranted. In fact, the tendency of prey to suppress their drift in the presence of *Rhyacophila* could lead to a negative predator impact (*sensu* Sih & Wooster 1994), i.e. higher prey densities in patches with than without predators. Finally, Soluk & Richardson (1997) have recently shown that stonefly predators may enhance the growth of stream-dwelling trout by inducing alterations in prey behaviour that make the prey more susceptible to trout. Our results indicate that such facilitation may, indeed, occur when fish live sympatrically with predatory stoneflies, but not when the predominant invertebrate predator uses a less mobile foraging mode, like, e.g. *Rhyacophila* larvae. Whether predator–predator facilitation is, indeed, an important mechanism supporting salmonid growth in streams may thus vary according to the relative abundances of invertebrate predators with contrasting foraging modes.

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