

Microhabitat and activity periodicity of predatory stoneflies and their mayfly prey in a western Colorado stream

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Experiments were conducted to determine whether overlap between microhabitat preferences and activity periodicities of four mayfly species and their stonefly predators could explain species-specific differences in predator-prey encounter frequencies. Preferences for rock type (slate or granite), flow microhabitat (high or low), rock surface (top, bottom, upstream or downstream sides), and periodicity of drift and the use of rock tops were measured in a stream-side system of flow-through circular Plexiglas chambers receiving natural stream water and light levels. These parameters were compared among the predatory stoneflies, *Megarcys signata* or *Kogotus modestus*, and four species of mayflies that vary in their encounter rates with the stoneflies. Based on predator-prey encounter rates previously observed in similar chambers, we expected greater overlap between *Megarcys* and *Ephemerella infrequens* and the overwintering generation of the bivoltine mayfly, *Baetis bicaudatus* than with *Cinygmula* sp. Likewise, we expected *Kogotus* microhabitat use to overlap more strongly with that of summer generation *Baetis* than with later instars of *Cinygmula* and *Epeorus deceptivus*. Results ran counter to our predictions, indicating that microhabitats of the prey species with high predator encounter rates did not overlap more strongly with the stoneflies than did mayflies with low predator encounter rates. Most mayflies and stoneflies preferred the bottom surfaces of granite rocks, and showed few flow preferences. Most were nocturnal in their use of top rock surfaces, in drift and feeding activity periodicity. Therefore, nocturnal activity periodicities of both mayflies and stoneflies confirm that mayflies have not evolved feeding periodicity to avoid encounters with foraging stonefly predators. We conclude therefore, that neither temporal nor spatial microhabitat overlap is a reasonable explanation of differential encounter rates between predatory stoneflies and their mayfly prey. Alternative explanations for differential encounter rates are that more abundant or more mobile mayflies have higher encounter rates with predators, and effective pre-contact predator avoidance responses of other mayflies reduce their encounter rates with stoneflies.

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Stream ecologists have been interested in the microhabitats and activity periodicity of benthic invertebrates mostly from the perspective of exposure to current and the potential of consequent vulnerability to being entrained in the drift (Elliott 1968, Bohle 1978, Wiley and Kohler 1980, Bailey 1981, Kohler 1983, 1985, Statzner

and Mogel 1985, Allan et al. 1986, 1991, Wilzbach 1990). Thus, the periodicity of invertebrate activity on the upper surfaces of substrates has been examined in an attempt to understand why the overwhelming majority of benthic invertebrate drift is nocturnal. Fewer investigators have studied invertebrate microhabitats or period-

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icity specifically from the perspective of predator-prey overlap or vulnerability of prey to predators in different microhabitats (Martin and Mackay 1983, Cooper 1985, Hershey and Dodson 1985, Holomuzki and Messier 1993). These studies have suggested that prey species may select microhabitats on the basis of risk of predation, residing most often in areas offering a refuge from predation.

Thus, reducing microhabitat overlap may be a highly effective prey defense, since differential encounter rates between predators and prey are often considered the most important explanation of selective predation in aquatic systems (e.g. Bergelson 1985, Cooper et al. 1985, Osenberg and Mittelbach 1989, Peckarsky et al. 1994). Similarly, prey may reduce encounter rates with predators by minimizing temporal overlap in activity periodicity. Alternative explanations for why some prey species or sizes are encountered by predators more frequently than others are: because they are more abundant, more mobile, more conspicuous, have poor pre-contact predator avoidance behavior. We have reported data on the influence of prey abundance, prey mobility, and prey behavior on stonefly-mayfly encounter rates elsewhere (Cooper et al. 1990, Peckarsky 1991, Peckarsky et al. 1994).

In this study, we have focused our attention on measuring predator-prey microhabitat and activity periodicity overlap under experimental conditions. Data obtained during field observations over three years in streams in western Colorado showed no consistent associations between predator or prey densities and any measured microhabitat variables, such as flow, water depth, substrate size or geologic origin, periphyton or detritus resource levels (Peckarsky 1991). We attribute this result to the complexity of potential interactive effects of many factors all of which play some role in determining distributions and abundances of organisms in nature. For this reason, we chose to investigate predator and prey microhabitats and activity periodicities under controlled experimental conditions, recognizing that caution should be exercised when extrapolating results of these tests to the natural environment.

In streams in western Colorado, we have demonstrated that predatory stonefly larvae, *Megarcys signata* and *Kogotus modestus* (Plecoptera: Perlodidae) feed selectively on *Baetis bicaudatus* (Ephemeroptera: Baetidae) larvae over all other species of mayflies available (Peckarsky and Penton 1989). Further, when foraging in chambers with natural substrates, stoneflies have higher encounter rates with *Baetis* than with most other mayflies in the system (Peckarsky et al. 1994). The purpose of this study was to investigate the microhabitat and activity periodicity overlap between alternative mayfly prey species and predatory stoneflies as a potential explanation for disproportionately high encounter rates on *Baetis*.

Methods

Microhabitat overlap experiments

Experiments were conducted in a stream-side system of circular Plexiglas flow-through chambers (described and illustrated in Peckarsky and Cowan 1991) that used natural stream water gravity-fed from a first order fishless tributary (Benthette Brook) of the East River, Gunnison County, Colorado, USA, at the Rocky Mountain Biological Laboratory. Water temperatures in the artificial streams reflected those of the adjacent East River, since water was piped through the East River for about 50 m before entering the streams located in a portable greenhouse (Weatherport). Water circulated in replicate stream chambers of 15 cm diameter, exited each unit through a mesh window in a central standpipe, and was collected in troughs through which it flowed back into the stream. Each chamber received contiguous substrates consisting of two slate and two granite 5-cm diameter rocks with their associated aufwuchs from the East River placed in the same position in the experimental chambers as they were in the stream, and situated randomly with respect to two water jets that directed the flow clockwise. The two rocks that were placed immediately downstream of the water jets had higher flows ($15.6 \pm 1.0 \text{ cm}^{-1}$) than those upstream of the jets ($6.3 \pm 0.5 \text{ cm}^{-1}$) measured with a Nixon micropropeller flowmeter at about 60% of the water depth, which was maintained at about 8 cm. Therefore, the chambers provided two substrate types (slate and granite) and two flow microhabitats (high and low).

Each chamber received either one predatory stonefly (*Megarcys* or *Kogotus*) or 8 mayflies of a single species. After at least one hour of acclimation (deemed sufficient by preliminary trials), observers recorded the position of each stonefly or mayfly six times over a diel cycle (1300, 1700, 2100 0100, 0500, and 0900 h) noting which rock and rock surface were occupied, whether stoneflies were foraging or whether mayflies were drifting within the chambers. Nighttime observations were made using dim red light, which was determined not to affect the behavior of all species, with the exception of one heptageniid mayfly species (*Cinygmula* sp.). Observations were made on three different occasions: 27–28 July, 1988 with *Kogotus* and one heptageniid species, *Epeorus deceptivus*; 19–21 June, 1989 with *Megarcys* and three different mayfly species (*Baetis*, *Cinygmula*, and one ephemereiid: *Ephemerella infrequens*); and 19–21 July, 1989 with *Kogotus* and *Baetis*, *Cinygmula* and *Epeorus*).

Species combinations were selected for concurrent observation based on their overlapping phenologies in samples taken from individual rocks collected in the East River (Peckarsky 1991), and thus, reasonable probability of predator-prey encounters. Since *Megarcys* emerges earlier in the summer than *Kogotus* (Peckarsky and Cowan 1991), the overwintering generation of bivoltine *Baetis* was tested with *Megarcys* in June and the

short summer generation of *Baetis* with *Kogotus* in July. In contrast, *Cinygmula* individuals tested with *Megarcys* in June were of the same generation, but earlier instars than individuals observed with *Kogotus* in July since this mayfly species is univoltine. Interestingly, *Epeorus* is absent from Benthette Brook; thus we conducted a pilot experiment (1988) in which slate and granite rocks were included in each chamber from both the East River and Benthette Brook to test whether this mayfly species preferred substrates from the East River. Replication for the 1989 experiments was 12 chambers per species, and $n = 10$ for the 1988 experiment.

The analyses fall into three general categories: habitat choices made among rocks (slate vs granite, East River vs Benthette Brook, high vs low flow), habitat choices made within rocks (top, bottom, upstream side and downstream side, which were delineated for individual rocks by noting the specific pattern of flow) and patterns of diel periodicity in positioning on stones and drifting in the water column (mayflies) or foraging (stoneflies). Paired t-tests (two-tailed) were conducted on means per chamber of percent mayflies exhibiting a certain response across time or on means per chamber of binomial responses (0, 1) for each stonefly across time. Wilcoxon signed rank tests were used whenever data were not normally distributed or able to be transformed to meet that criterion. Differences in diel periodicity of use of rock surfaces were tested by comparing combined observations at 0900, 1300 and 1700 h (diurnal) to combined observations at 2100, 0100 and 0500 h (nocturnal), because examination of day and night patterns of distribution and behavior indicated that pooling those data was appropriate. Percentages for habitat choices among rocks were based on the total number of individuals actually observed on the rocks. For habitat choices within rocks, the numbers not observed were assumed to be on the bottoms, and were not included in the among-rock analysis. The percent mayflies drifting or number of stoneflies foraging were based on the total number of individuals in the chamber. Each chamber was considered an independent unit, since chemicals from predator tanks could not have affected prey behavior in adjacent tanks or vice versa.

Periodicity of gut fullness

To supplement the positioning periodicity data we also collected larvae of each mayfly species from the East River and analyzed their feeding periodicity. Twelve larvae were obtained from benthic samples in a single riffle at 3–4 h intervals over a 24-h cycle in August 1990 (*Epeorus*), July 1991 (*Cinygmula*), and June–July 1992 (*Ephemerella*). (Data on *Baetis* feeding periodicity have been published previously, Cowan and Peckarsky 1994). Upon sampling, animals were placed individually in amber bottles containing several ml of 90% ethanol, and gut fullness was determined fluorometrically (as in

Cowan and Peckarsky 1990). Gut pigments were extracted from macerated animals, and the fluorescence of the extracts was read on a Turner Model 112 fluorometer. Fluorescence of macerated animals was corrected for pigments associated with the exoskeleton and not the gut by determining species-specific fluorescence of the body after the gut had been removed by dissection. Most species had very little background body fluorescence (*Baetis*: 4.1 ± 2.3 ng, *Epeorus* *deceptivus*: 7.3 ± 0.8 ng; *Cinygmula*: 8.7 ± 1.0 ng; but *Ephemerella* exoskeletons accumulated substantial amounts of pigments (323 ± 181 ng). Gut fullness was expressed as ng total pigment (chlorophyll *a* + phaeopigment *a*) per individual, which was a more accurate estimate of the amount of algal material in the gut at the time of sampling, because chlorophyll degraded very rapidly in the gut to phaeopigment (Cowan and Peckarsky 1990). Data were analyzed by two-sample t-test (two-tailed) comparing log-transformed night vs day gut fullness on each species.

Gut clearance time

Inferences about feeding rates can be made from gut

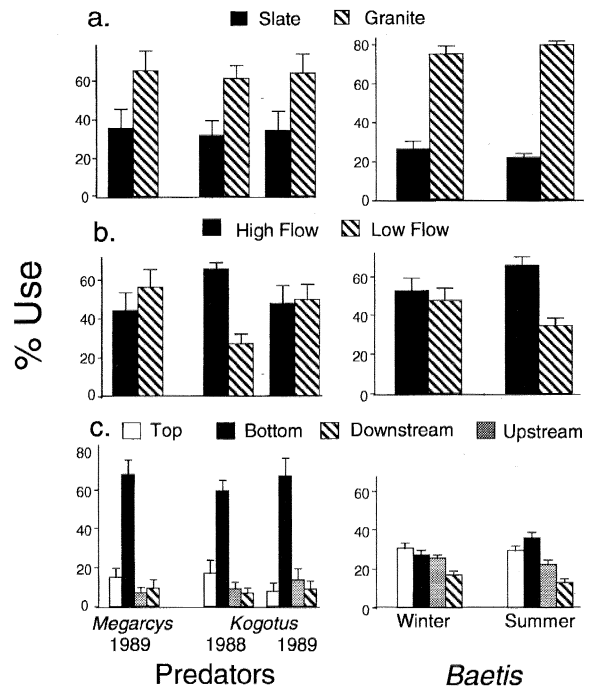


Fig. 1. Microhabitat preferences of predators and preferred prey: mean \pm 1 SE percent use of different microhabitats by predatory stoneflies (left), *Megarcys signata* (1989) and *Kogotus modestus* (1988, 1989), and *Baetis bicaudatus* (right), overwintering generation (June 1989 concurrent with *Megarcys*), and summer generation (July 1989 concurrent with *Kogotus*). a. Rock type: slate or granite; b. Flow: high or low; c. Rock surface: top, bottom, upstream or downstream sides. Results of paired t-tests and associated probabilities for significant microhabitat preferences are given in text ($n = 12$).

Table 1. Microhabitat preferences of stoneflies and mayflies measured in circular flow-through chambers with algae-covered rocks. (First number in parentheses = paired T statistic, second number = p. If only one number in parentheses = p from non-parametric test.)

Predators	Trial	Rock type	Flow	Rock surface	Periodicity		
					use of rock tops	activity ^a	feeding ^b
<i>Megarcys</i>	June 1989	no pref. (1.44, .177)	no pref. (-.64, .536)	bottom (.005)	nocturnal (.010)	nocturnal (3.07, .011)	nocturnal
<i>Kogotus</i>	July 1988	granite (2.14, .061)	high (4.76, .001)	bottom (.017)	nocturnal (.050)	nocturnal (1.81, .104)	nocturnal
	July 1989	no pref. (1.46, .173)	no pref. (-.10, .925)	bottom (.006)	aperiodic (.547)	nocturnal (4.02, .002)	
Prey							
<i>Baetis</i> ^c	June 1989	granite (5.97, .001)	no pref. (.41, .690)	avoided downstream (.008)	nocturnal (4.39, .001)	nocturnal (7.10, .001)	nocturnal
	July 1989	granite (14.11, .001)	high (3.67, .004)	avoided downstream (.004)	aperiodic (.41, .693)	nocturnal (3.96, .002)	weakly noct.
<i>Cinygmula</i>	June and July 1989	no pref. (1.74, .110)	no pref. (-.30, .767)	bottom (.002)	nocturnal (5.64, .001)	nocturnal (4.16, .002)	aperiodic (?) (.530)
	July 1991	(1.36, .202)	(-.27, .780)	(.002)	(4.20, .002)	(2.97, .013)	
	<i>Epeorus</i>	July 1988	slate (2.61, .029)	no pref. (1.07, .315)	bottom (.009)	nocturnal (4.58, .001)	
<i>Epeorus</i>	July 1989	no pref. (-.38, .714)	no pref. (1.10, .296)	bottom (.002)	nocturnal (2.90, .015)	nocturnal (2.56, .027)	nocturnal (?) (0.046)
	Aug. 1990						
<i>Ephemera</i>	June 1989	granite (2.15, .055)	high (1.98, .074)	bottom (.002)	aperiodic (1.67, .123)	aperiodic (.76, .464)	aperiodic (.531)
	June 1992						

^a Predators: activity = foraging (crawling over all rock surfaces). Prey: activity = drift in the water column.

^b Feeding periodicity of predators from Martinez and Peckarsky (1989), and *Baetis* from Cowan and Peckarsky (1994). (?) indicates that slow gut clearance time reduces resolution of method.

^c June experiments on overwintering generation *Baetis*, July experiments on summer generation *Baetis*.

fullness data only if the gut clearance time is known, because ingestion rate is the product of instantaneous gut fullness and the gut clearance rate constant (Boyd and Smith 1980, Dagg and Wyman 1983). If the sampling interval in diel feeding periodicity studies is less than the gut clearance time of the study animal, full guts may simply be an artifact of slow clearance rates rather than recent feeding (Mackas and Bohrer 1976). Thus, to facilitate interpretation of feeding periodicity data, gut clearance rates were measured in the circular stream system for all mayfly species. We collected 96 larvae of each mayfly species at a time during the 24-h cycle when guts were most full (as determined by methods described above), and placed them in circular streams containing rocks that had been boiled and scrubbed to remove algae. We then removed 12 larvae at various intervals of starvation (0, 0.5, 1, 2, 4, 8, 12, and 24 h past time of collection) and analyzed their guts fluorometrically to determine the rate at which photosynthetic pigments (ng per individual) disappeared (gut clearance time).

Results

Microhabitat overlap experiment

Although both predator species rested on granite rocks more often than slate rocks (Fig. 1a left), this trend was not significant for *Kogotus* in the 1988 experiment (Table 1). Since *Kogotus* did not distinguish between rocks from the East River and Benthette Brook ($T = 0.10$, $p = .920$), results for rock types were pooled. *Kogotus* preferred high flow rocks in 1988 only, but *Megarcys* showed no preferences for different flow microhabitats (Fig. 1b left, Table. 1). All stoneflies rested on rock bottoms in preference to rock tops (Fig. 1c left, Table. 1), and showed distinct nocturnal peaks in foraging activity (Fig. 2a left, Table. 1), at which time they cruised around the chambers from rock to rock in search of prey. This foraging behavior resulted in significant nocturnal periodicity in use of rock tops for both *Megarcys* and *Kogotus* (Fig. 2b left, Table. 1).

Mayfly species and different cohorts or instars of the same species differed in their responses among rocks

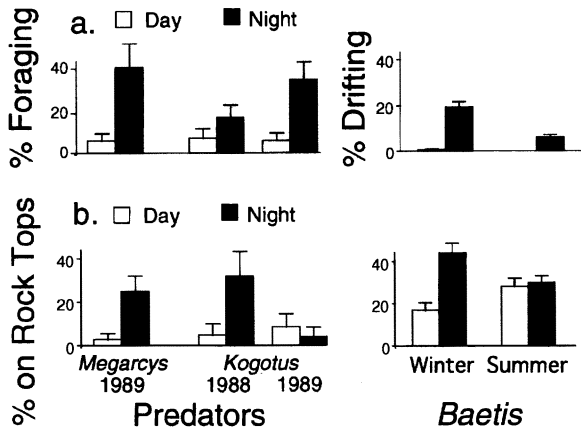


Fig. 2. Activity periodicity of predators and preferred prey. a. Mean + 1 SE foraging activity (percent foraging) by day and night of predatory stoneflies (left), *Megarcys signata* (1989) and *Kogotus modestus* (1988, 1989), and percent drifting day and night of *Baetis bicaudatus* (right), overwintering generation (June 1989 concurrent with *Megarcys*), and summer generation (July 1989 concurrent with *Kogotus*). b. Mean + 1 SE percent use of rock tops during day and night by predators (left) and by *Baetis* (right). Results of paired t-tests and associated probabilities for significant periodicities are given in text (n = 12).

and within rocks, and in their activity periodicities (Table 1). Both generations of *Baetis* clearly preferred granite rocks (Fig. 1a right), but only summer generation *Baetis* selected high flow microhabitats (Fig. 1b right). Individuals of both generations were evenly distributed among tops, upstream sides and bottoms of rocks, but had lower abundance on downstream rock faces compared with rock tops (Fig. 1c right). Drift periodicity of both generations of *Baetis* was nocturnal (Fig. 2a right). Individuals of the overwintering generation were nocturnal in their use of upper rock surfaces, but summer generation *Baetis* was aperiodic in use of rock tops (Fig. 2b right). Thus, the two cohorts of *Baetis* often differed in their microhabitat preferences (Table 1), which could be attributed to size differences between the cohorts or to behaviors relating to growth rate and avoidance of trout predation (Cowan and Peckarsky 1994).

In contrast, early and late instars of *Cinygmula* were consistent (Table 1), showing no preferences for rock type or flow microhabitat, but a strong preference for bottoms of rocks (Fig. 3a, b, c left). Also, the use of rock tops and drift periodicity of early and late instars were consistently nocturnal (Fig. 4a, b left). Responses of *Epeorus* were generally consistent between years (Table 1), except in their preferences for rock type (Fig. 3a center). In the 1988 experiment *Epeorus* preferred slate over granite rocks, and tended to use East River rocks more than Benthette Brook rocks, but this trend was not significant ($T = 1.33$, $p = 0.217$). In 1989, however, *Epeorus* showed no preference for rock type. In both years, this species had no preferences for flow microhabitat (Fig. 3b center), preferred rock bottoms to sides (Fig. 3c center), and was nocturnal in use of upper rock surfaces

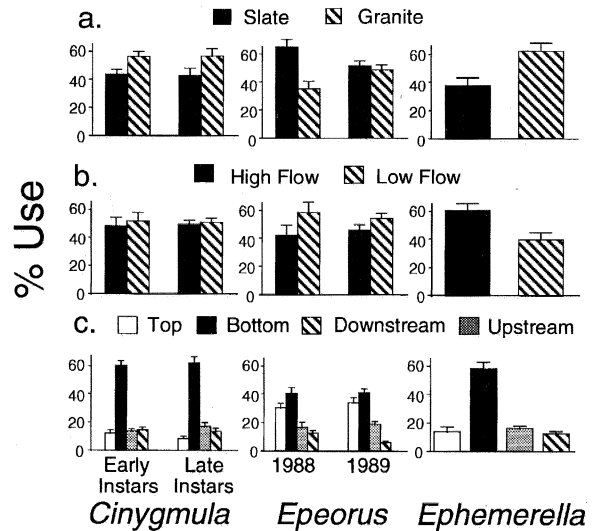


Fig. 3. Microhabitat preferences of mayflies: mean + 1 SE percent use of different microhabitats by *Cinygmula* sp. (left), early instars (June 1989 concurrent with *Megarcys*), and late instars (July 1989 concurrent with *Kogotus*); *Epeorus* *deceptivus* (center), (July 1988 and 1989 concurrent with *Kogotus*); and *Ephemerella infrequens* (right), (June 1989 concurrent with *Megarcys*) a. Rock type: slate or granite; b. Flow: high or low; c. Rock surface: top, bottom, upstream or downstream sides. Results of paired t-tests and associated probabilities for significant microhabitat preferences are given in text (n = 12).

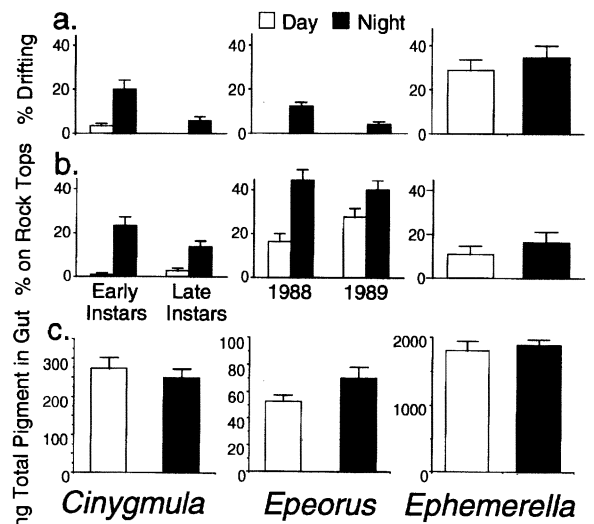


Fig. 4. Activity periodicity of mayflies: mean + 1 SE percent drifting (a), percent using rock tops (b) and total gut pigments (chlorophyll *a* and phaeopigments) (c) day and night of *Cinygmula* sp. (left), early instars (June 1989 concurrent with *Megarcys*), and late instars (July 1989 concurrent with *Kogotus*); *Epeorus* *deceptivus* (center), (July 1988 and 1989 concurrent with *Kogotus*); and *Ephemerella infrequens* (right), (June 1989 concurrent with *Megarcys*). Note different axes on gut content plots of each mayfly species. Results of paired t-tests and associated probabilities for significant periodicities are given in text (n =

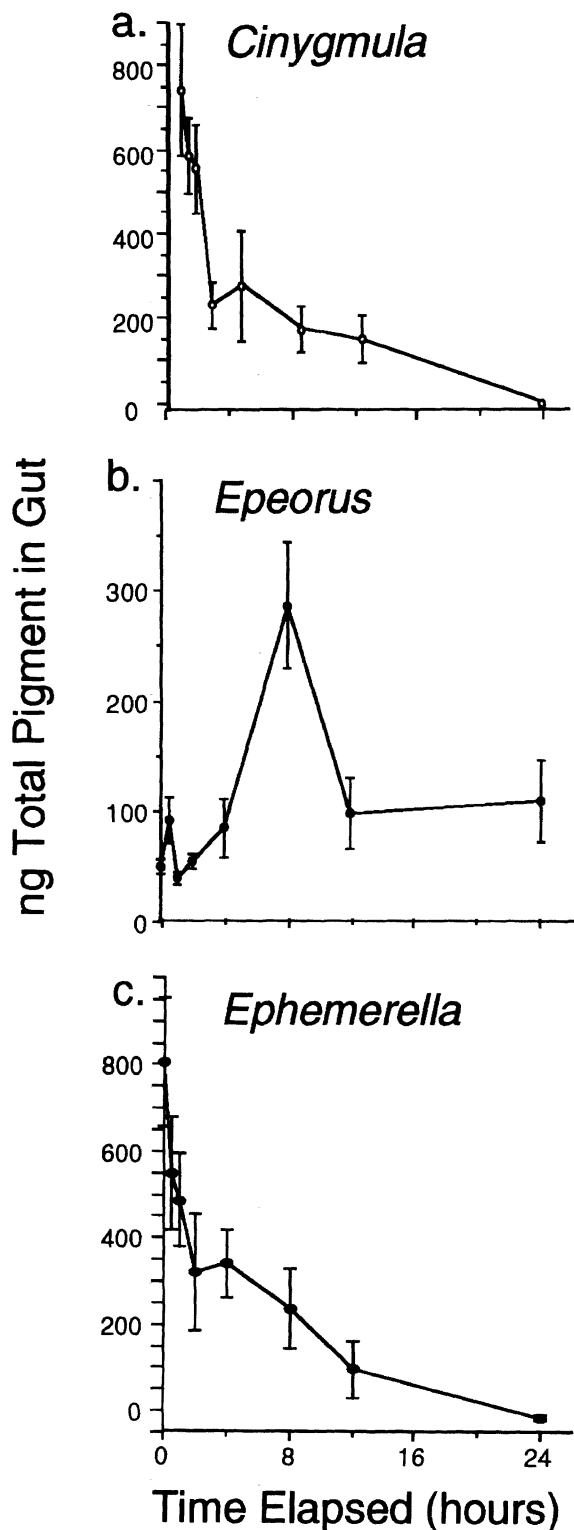


Fig. 5. Gut clearance time: mean \pm 1 SE ng total pigment (chlorophyll *a* + phaeopigment) per mayfly individual at intervals of 0–24 h confined with no food. a. *Cinygmula* sp., b. *Epeorus deceptivus*, c. *Ephemerella infrequens*.

(Fig. 4b center). Drift periodicity of *Epeorus* was also nocturnal in both the 1988 and 1989 experiments (Fig. 4a center). Finally, *Ephemerella* preferred granite over slate rocks (Fig. 3a right), had a weak preference for high flow rocks (Fig. 3b right), chose rock bottoms over other faces (Fig. 4c right), and were aperiodic in their use of rock surfaces and drift periodicity (Fig. 4a, b right).

Periodicity of gut fullness

Feeding periodicity, as indicated by ng photosynthetic pigments per gut, was not always consistent with activity periodicities and uses of stone surfaces (Fig. 4, Table 1). *Baetis* feeding periodicity differed between overwintering and summer generations (Table 1, Cowan and Peckarsky 1994). The overwintering generation was strongly nocturnal, whereas the summer generation was only weakly nocturnal, consistent with its more aperiodic use of rock surfaces shown in this study (Fig. 2b right, Table 1). Feeding periodicity data for this species can be measured reliably using the fluorometric technique, because *Baetis* guts were clear of pigment in 2–4 h, even at very low stream temperatures (Cowan and Peckarsky 1994). In contrast, this technique was less reliable for all other mayfly species, because gut clearance times were much slower (Fig. 5), resulting in a loss of resolution of the source of pigments measured in the guts at each time in the diel cycle.

Cinygmula guts indicated aperiodic feeding (Fig. 4c left), in contrast to their nocturnal use of stone surfaces and nocturnal drift periodicities (Figs 4a, b left, Table 1). These data may be a questionable indicator of feeding periodicity, because although *Cinygmula* cleared the majority of pigment from its guts in about 2 h, guts were not completely cleared until 24 h (Fig. 5a) and the amount of pigment remaining in the guts after 4 h was similar to that in the guts of individuals used for the feeding periodicity trials (Fig. 4c left). Whereas *Epeorus* guts indicated nocturnal feeding (Fig. 4c center) consistent with their nocturnal use of rock surfaces and drift periodicity (Figs 4a, b center, Table 1), their guts did not clear of pigment even over a 24-h period (Fig. 5b). Therefore, data on periodicity of gut fullness may also not be reliable for this species. Finally, *Ephemerella* gut analysis indicated aperiodic feeding (Fig. 4c right), consistent with their use of rock surfaces and drift periodicity (Figs 4a, b, Table 1). These data may be more reliable than those of the heptageniids, because although *Ephemerella* did not clear their guts completely until 24 h (Fig. 5c), after 1 h the amount of pigment present was reduced to about 20% of that in the guts of the individuals tested in the periodicity trial (Fig. 4c right).

Discussion

Data on microhabitat preferences and activity periodicities can be interpreted in light of differential encounter frequencies between stoneflies and their prey to determine whether overlap or lack thereof are responsible for those differences. Experiments measuring predator-prey encounter rates (defined as contacts) on natural substrates showed that *Baetis* and *Ephemerella* have significantly higher encounter rates with stoneflies than do heptageniid mayflies (*Epeorus* and *Cinygmula*) offered in equal densities in experimental streams (Peckarsky et al. 1994). We know that *Baetis* and *Ephemerella* are more mobile than heptageniids (Cooper et al. 1990), and that *Baetis* is the most abundant mayfly in the system, followed closely by *E. deceptivus*, and distantly by all other species (Peckarsky 1991). This study was designed to determine whether the microhabitats and activity periodicities of predators and potential prey (in the absence of encounters) were consistent with the observed differences in predator-prey encounter rates. Other experiments that tested the microhabitat preferences of prey species in the presence of predators demonstrate that microhabitats occupied by mayflies were not significantly affected by the presence of predatory stoneflies (Peckarsky in press). Therefore, we can interpret the results of data obtained here relative to the more natural situation where predators and prey coexist.

With one exception (*Epeorus* 1988), higher encounter rates by stoneflies on *Baetis* and *Ephemerella* vs the heptageniids cannot be explained by overlap of predator and prey preferences for rock type (Table 1). All other mayfly species and both stonefly species were either found more often on granite than slate rocks, or showed no preference for rock types. The reasons for those preferences were not determined in this study, although we speculate that granite provides more complex texture and perhaps constitutes a better refuge from flow providing more interstitial space between rocks and the walls of the chambers. Other studies have demonstrated that mayflies prefer substrates with more complex texture (Erman and Erman 1984, Casey and Clifford 1989, Sanson et al. 1995). Further, diatoms may be less prone to scouring from rough granite surfaces compared to smooth slate surfaces, which could affect the availability of algal resources for some grazers. Although we have not measured higher chlorophyll levels or differences in diatom species composition between these two rock types (Penton, M. A., unpubl.), Sanson et al. (1995) report a positive association between substrate roughness and chlorophyll *a*.

The one exception to this pattern was that *Epeorus* showed a preference for slate rocks in the 1988 experiment, which could have reduced their encounter rates with the predator, *Kogotus*, which preferred granite rocks in the same experiment. Other studies suggest that *Epeorus* may prefer slate rocks due to their mouthparts being better adapted to removal of diatoms from smooth

surfaces (Oglivie 1988, Padilla, Penton and Peckarsky, unpubl.). Further, the absence of *Epeorus* from Benthette Brook may be due in part to the rarity of slate rocks and their associated algal resources in this stream (75% granite, 17% slate) compared to the East River (33% granite, 50% slate).

Similarly, predator and prey flow microhabitat preferences were not consistent with differences in predator-prey encounter rates (Table 1). Most species did not differentiate between flow microhabitats, with the exception that summer generation *Baetis*, *Ephemerella*, and *Kogotus* preferred high flow rocks. But this observation is not sufficient to explain high encounter rates between stoneflies (especially *Megarcys*) and *Baetis* and *Ephemerella*. Flow microhabitats were also different depending on which rock surface was inhabited. Highest flow rates were measured on the upstream side and tops; and flows could actually be quite high on the upstream edge of the rock bottoms (unpubl.). However, lowest flow microhabitats existed on the downstream faces of rocks. Interestingly, this microhabitat was avoided by most species of mayflies and stoneflies, which primarily preferred rock bottoms, and in the case of *Baetis*, all rock faces except the downstream sides. All species of mayflies overlapped strongly with stoneflies in their preference for rock bottoms. Further, stoneflies only resided on rock bottoms when at rest, and searched all rock faces and types when foraging in the chambers. Therefore, preference by mayflies for specific rock types or locations on rocks did not constitute a refuge from stonefly predation.

Periodicity in the use of rock tops, drift, or foraging activity was largely nocturnal for all species, with the exception of *Ephemerella* and *Kogotus* in one of two experiments. These data are consistent with those of other accounts of stonefly foraging periodicity (Malmqvist and Sjöström 1980, Johnson 1981, 1983, Martinez and Peckarsky 1989). Walde and Davies (1985) similarly showed that *Kogotus* is sometimes day active. But for the most part, predatory stonefly foraging behavior is largely crepuscular or nocturnal. The observation of overwhelming increases in mayfly activity during the same periods that stoneflies are active strongly suggests that avoidance of stonefly predation was not the selection pressure causing the evolution of this nocturnal periodicity. Alternatively, such increases in nocturnal activity of stream invertebrates have been attributed to constraints on foraging behavior due to avoidance of visually feeding predatory fish (Allan 1978, Flecker 1992, Culp and Scrimgeour 1993, Cowan and Peckarsky 1994, Douglas et al. 1994), or to redistribution in response to unfavorable abiotic conditions, such as low flows and/or dissolved oxygen levels (Kovalak 1978, 1979, Wiley and Kohler 1980).

Many other studies have demonstrated nocturnal drift periodicity in mayflies (see review of Waters 1972), or nocturnal increases in the use of rock tops by mayflies (Elliott 1968, Glozier and Culp 1989, Rader and Ward

1990). But as in this study, drift periodicities were not always concordant with activity on rock tops (Bailey 1981, Kohler 1983, Graesser and Lake 1984, Statzner and Mogel 1985, Allan et al. 1986, Casey 1987, Wilzbach 1990). Measurements used to assess drift, positioning and feeding periodicities often have different resolution, making them difficult to compare. In this study, drift and positioning periodicities were measured in the same experimental chambers. Therefore, non-concordance of these phenomena can be attributed to a lack of association between them (only observed for summer generation *Baetis*, which was aperiodic in its use of substrate surfaces, yet exhibited nocturnal drift periodicity).

However, non-concordance of gut fullness periodicity with behavioral observations (which occurred in *Cinygmula*) could be attributed to differences in resolution of the methods used to determine feeding periodicities. For example, in two separate studies, Allan et al. (1986, 1991) demonstrated different activity periodicities for *Baetis* and *Cinygmula* using different methods of observation (human observers using dim red light vs time-lapse cinematography with periodic flashes). Heise (1992) and Johansson (1992) provide elegant demonstrations that simulating darkness with dim red light may be inappropriate when studying behavior of organisms with well-developed eyes. In the present study, we would advise caution when interpreting the behavioral data from nighttime observations of *Cinygmula* for this reason. This mayfly was sensitive to illumination by dim red light, which could have biased our observations of its microhabitat and activity periodicity. Given the uncertainty of these behavioral data as well as the problems with slow gut clearance time, we cannot draw reliable conclusions on the periodicity of this mayfly species. However, observations made on all other species using dim red light did not affect their behavior (Palmer in press).

In summary, the observed preferences of mayfly species for specific rock types, flow microhabitats and rock surfaces were not consistent with the hypothesis that species overlapping more extensively with predatory stoneflies are encountered with higher frequencies. Further, nocturnal activity periodicities of both mayflies and stoneflies confirm that mayflies have not evolved feeding periodicity to avoid encounters with foraging stonefly predators. We conclude from the available data that neither temporal nor spatial microhabitat overlap is a reasonable explanation of differential encounter rates between predatory stoneflies and their mayfly prey. Alternative explanations for differential encounter rates are that more abundant (e.g., *Baetis*, Peckarsky 1991) or more mobile (e.g., *Baetis* and *Ephemerella*, Cooper et al. 1990) mayflies have higher encounter rates with predators, and effective pre-contact predator avoidance responses of some mayflies (e.g. heptageniids, Peckarsky et al. 1994) reduce their encounter rates with stoneflies. The present study indicates that microhabitat preferences given in the experimental chambers offered no ad-

ditional refuge from stonefly predation. Our results do not rule out, however, the possibility that predators and prey select different microhabitats in the field that we were unable to provide in our experimental system. Further tests of microhabitat overlap in more complex experimental arenas and at multiple temporal and spatial scales are warranted.

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