

THE IMPORTANCE OF SPECIES ASSOCIATIONS AND SUBSTRATE TYPES TO BEHAVIOURAL DRIFT

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ABSTRACT

Laboratory experiments were conducted to study drift responses of two herbivorous mayflies and a carnivorous stonefly with different substrate types. Light was the only factor studied that influenced drift of immature *Baetis* sp.* nymphs. The predator, *Isogenoides elongatus* (Hagen), significantly influenced the drift of both mature *Baetis* sp. and *Leptophlebia cupida* (Say) nymphs. With the presence of artificial vegetation, *L. cupida* nymphs drifted in much lower numbers. The presence of *Leptophlebia* nymphs significantly influenced drift of *I. elongatus* specimens during dark periods. The two cohabiting herbivorous species showed no increased drift response to one another. It is suggested that during daylight hours there is a greater risk of predation for mature *Baetis* nymphs to enter the water column than to remain on the stream bed.

INTRODUCTION

Several workers have shown that some organisms have a greater propensity to drift than others (Waters 1969, Lehmkuhl and Anderson 1972, Corkum 1978). Most drifting animals reported in the literature are detritivores or herbivores, whereas invertebrate predators are only infrequently present in the drift (Waters 1969, Adamus and Gauhin 1976). Although changes in light intensity significantly influence the periodicity of drifting organisms (Waters 1972), perhaps some portion of the drift fauna is also avoiding benthic invertebrate predation.

* *Baetis* sp. is now known to be *Baetis tricaudatus* Dodds.

Corkum (1978) has shown a difference between swimming and crawling ephemeropteran nymphs in their tendency to leave the substrate and enter the water column. In this study, we suggest that these behavioural groups have different methods or strategies of avoiding predators. Will swimmers or crawlers more likely drift or seek cover to avoid predation? Since the behaviour of a nymph may also change with size, mature and immature nymphs of the same species may avoid predators in different ways.

Hildebrand (1974) has shown that reduced food levels tend to increase drift in some mayfly species. Although mayfly nymphs are frequently associated with macrophytes (Barber and Kevern 1973), plant cover has not been adequately examined as a factor influencing drift. By utilizing different substrate types (food and cover) and different organisms associations (herbivores and predators), we attempted to determine the factors that most influence the drift of the mayflies *Baetis* sp. and *Leptophlebia cupida* (Say) and the stonefly *Isogenoides elongatus* (Hagen).

METHODS

The Pembina River, near Entwistle, Alberta (53°36'N, 115°00'W), served as a source of *Baetis* sp. and *I. elongatus* nymphs used in the laboratory experiments. There are few *L. cupida* nymphs in the Pembina River; hence, we collected these nymphs from the Bigoray River (53°31'N, 115°26'W). The Bigoray River flows into the Pembina upstream from the Entwistle site. Nymphs were collected no earlier than 2 days before any experiment and nymphs were never utilized in more than one experiment. Predators were starved for 24 h before each run.

Experiments were conducted in an artificial stream housed in an environmental chamber. A motor-driven paddlewheel within the stream provided a current of 10 cm/s; water depth was 15 cm. Two substrate trays (each 28 x 17.5 x 2.5 cm) positioned in the stream contained one of four substrates: (1) stones (16-23 mm in diameter), (2) stones + food, (3) stones + cover, and (4) stones + food + cover. Prepared instant oatmeal (40 g) was rubbed over wet substrate surfaces just before the trays were inserted into the stream. This procedure ensured that food sources were restricted to the substrate trays. Artificial turf was used for cover.

Nymphal sample sizes were 100 *Baetis* sp., 100 *L. cupida* and 10 *I. elongatus*. Depending on the trial, nymphs of one or more species were poured through a funnel into the raceway upstream from the substrate trays. The nymphs suspended in the water column were counted for 10 s each minute for 45 min as they passed a reference point in the stream. Afterwards, a net with attached jar was inserted immediately downstream from the substrate trays. For the duration of the experiment (3 h of light, followed by 3 h of dark), the net was

examined at 30-min intervals, at which time any drifting organisms were removed and preserved. Later, the foregut of each predator was examined.

From 28 September to 22 October 1977 (autumn experiments), experiments were carried out with immature *Baetis* sp. (Clifford's 1970, stage II nymphs) using a 2 x 2 x 2 x 2 factorial design (presence/absence of predators, food, cover and light). Three trials were conducted. Recordings of settling, however, were only entered for two trials. Water temperature was 5°C.

From 21 April to 25 May 1978 (spring experiments), experiments were conducted with mature mayflies (Clifford's 1970, stage III nymphs). The set-up for both *Baetis* and *L. cupida* was a 2 x 2 x 2 x 2 x 2 factorial design (presence/absence of predators, food, cover, light, and the second mayfly species). For *I. elongatus*, the set-up (2 x 2 x 2 x 2 x 2) represented the presence/absence of *Baetis* sp., *L. cupida*, food, cover and light. These spring experiments were performed twice. Water temperatures ranged from 5 to 10°C depending on field conditions.

RESULTS

Nymphal Number Suspended in the Water Column Versus Time

For all mature nymphs of *Baetis*, *L. cupida* and *I. elongatus*, 95% of each sample population settled onto all substrate types within 5 min of the nymphs being added to the stream. Immature *I. elongatus* nymphs (autumn experiments) also quickly settled onto the substrate. In contrast, immature *Baetis* nymphs tended to remain longer within the water column.

Regression equations were calculated for the natural logarithm of numbers of immature *Baetis* nymphs suspended in the water column versus time. In experiments both with and without predators, immature *Baetis* nymphs significantly differed in their ability to settle on the stream bed with different substrate types (Table 1). The nymphs settled more quickly when either food alone or food and cover were present on the stream bottom. It is likely that the immature *Baetis* nymphs actually became stuck to the oatmeal on the stream bed. With other substrate types, nymphs settled briefly, but then re-entered the water column. Immature *Baetis* nymphs were slowest in settling onto bare stones in the absence of a predator. For the other substrates, there was no significant difference in settling times with or without predators on the stream bed (Table 2).

Table 1. Significance of regression and analysis of covariance of the number of immature *Baetis* nymphs suspended in the water column versus time for all substrate types, with and without predators.

Equation format: $Y = Ae^{-BX}$

| Factors* | A | B (\pm 95% conf.) | Reg (p) | Covariance | |
|----------|-------|----------------------|---------|-------------------------|---------|
| | | | | Test of Slopes F_s | p |
| IBOS | 30.81 | 0.171 \pm 0.666 | < 0.001 | | |
| IBOSF | 43.54 | 0.761 \pm 0.317 | < 0.01 | 10.584 | < 0.001 |
| IBOSC | 35.96 | 0.236 \pm 0.121 | < 0.01 | | |
| IBOSFC | 59.46 | 0.783 \pm 0.302 | < 0.05 | | |
| IBPS | 52.18 | 0.344 \pm 0.114 | < 0.001 | | |
| IBPSF | 56.32 | 0.960 \pm 0.360 | < 0.05 | 4.950 | < 0.05 |
| IBPSC | 46.89 | 0.423 \pm 0.179 | < 0.01 | | |
| IBPSFC | 48.48 | 0.594 \pm 0.263 | < 0.05 | | |

*Code for factors: IBO: Immature *Baetis* with no predator
 IBP: Immature *Baetis* with predator
 S: stones
 SF: stones + food
 SC: stones + cover
 SFC: stones + food + cover

Table 2. Significance of analysis of covariance of immature *Baetis* with and without predators for each substrate type.

| Factors* | Covariance | | | |
|-----------------|----------------|--------|--------------------|------|
| | Test of Slopes | | Test of Intercepts | |
| | F_s | p | F_s | p |
| IBOS & IBPS | 5.840 | < 0.05 | 0.504 | n.s. |
| IBOSF & IBPSF | 0.648 | n.s. | 0.224 | n.s. |
| IBOSC & IBPSC | 2.693 | n.s. | 1.676 | n.s. |
| IBOSFC & IBPSFC | 0.844 | n.s. | 0.440 | n.s. |

*See footnote of Table 1 for explanation of factors.

Drift Experiments

A 4-way analysis of variance (ANOVA) was used to determine the influence of predators, cover, food and light on the drift of immature *Baetis* nymphs. There were significantly more nymphs caught in the drift net during daylight hours ($p < 0.001$). No other factors were significant. These results were consistent even when we eliminated the first half hour of the light period, which probably contained nymphs still suspended in the water column.

A 5-way ANOVA was used to determine the factors influencing the drift of mature *Baetis*, *L. cupida* and *I. elongatus* nymphs (Table 3). The presence of the stonefly predator, *I. elongatus*, significantly increased the drift of *Baetis* ($p < 0.001$) and, to a lesser extent, *L. cupida* ($p < 0.05$). When stoneflies were present, more than twice as many *Baetis* nymphs drifted than did nymphs of *L. cupida*. When both herbivorous species were present on the stream bed at the same time, neither species influenced the drift of the other. Even the active crawling behaviour of *L. cupida* did not significantly affect the numbers of drifting *Baetis* nymphs. In trials with both *Baetis* and *L. cupida*, 100 nymphs of each species were present in the stream. Even though the benthic density was doubled, no increase in drift for either species occurred.

The absence of vegetative cover significantly increased the numbers of drifting *L. cupida* nymphs ($p < 0.001$). Even though predators significantly influenced the drift of *L. cupida* from all substrate types, fewer *L. cupida* nymphs drifted from substrates with vegetation.

Both the presence of the crawling mayfly *L. cupida* and the absence of light significantly influenced the drift of the stonefly predator ($p < 0.001$). Various second, third and fourth order interactions also significantly affected the drift of the three species (Table 3). Food alone had no effect on the drift of any species.

Foregut Analysis

Foregut analysis of *I. elongatus* from laboratory studies showed that the predator ate nymphs of each mayfly species under both light and dark conditions. To determine which species was most often captured, only those predators still remaining on the substrates at the end of each experiment were analysed statistically. Since the total surface area of mature *L. cupida* nymphs is 2.8 times that of mature *Baetis* nymphs, the larger species would be three times more likely to be encountered than the smaller one (data on total body lengths and head capsule widths are given in Table 4). From chi-square analysis, we cannot reject this 3:1 ratio of predator encounter with the two mayfly species. There might be a tendency for this tactile predator to have more success in capturing crawling nymphs (*L. cupida*).

Table 3. Significant factors and their interactions influencing the drift of mature *Baetis* (3A), *Leptophlebia cupida* (3B), and *Isogenoides elongatus* (3C).

Table 3A - *Baetis* sp. Leach
(5-way ANOVA: *L. cupida*, *I. elongatus*, Cover, Food, Light)

| Factor | df | SS | MS | F | p |
|--|----|---------|---------|--------|---------|
| <i>I. elongatus</i> | 1 | 2.64736 | 2.64736 | 58.441 | < 0.001 |
| <i>I. elongatus</i> x Food | 1 | 0.27262 | 0.27262 | 6.018 | < 0.025 |
| Cover x Light | 1 | 0.22806 | 0.22806 | 5.034 | < 0.05 |
| <i>L. cupida</i> x Cover x Light | 1 | 0.28259 | 0.28259 | 6.238 | < 0.025 |
| <i>L. cupida</i> x <i>I. elongatus</i> x Light | 1 | 0.26402 | 0.26402 | 5.828 | < 0.025 |
| <i>I. elongatus</i> x Cover x Light | 1 | 0.36116 | 0.36116 | 7.973 | < 0.01 |
| <i>L. cupida</i> x <i>I. elongatus</i> x Cover x Light | 1 | 0.31043 | 0.31043 | 6.853 | < 0.025 |
| Error | 31 | 1.40441 | 0.04530 | | |
| Total | 63 | 6.92894 | 0 | | |

Table 3B - *Leptophlebia cupida* (Say)
(5-way ANOVA: *Baetis* sp., *I. elongatus*, Cover, Food, Light)

| Factor | df | SS | MS | F | p |
|------------------------------------|----|---------|---------|--------|---------|
| <i>I. elongatus</i> | 1 | 0.43992 | 0.43992 | 4.425 | < 0.05 |
| Cover | 1 | 2.70563 | 2.70563 | 27.217 | < 0.001 |
| <i>I. elongatus</i> x Food | 1 | 0.56063 | 0.56063 | 5.640 | < 0.025 |
| <i>I. elongatus</i> x Food x Cover | 1 | 0.61363 | 0.61363 | 6.173 | < 0.025 |
| Error | 31 | 3.08172 | 0.09941 | | |
| Total | 63 | 6.92894 | 0 | | |

Table 3C - *Isogenoides elongatus* (Hagen)
(5-way ANOVA: *Baetis* sp., *L. cupida*, Cover, Food, Light)

| Factor | df | SS | MS | F | p |
|----------------------------------|----|---------|---------|--------|---------|
| <i>L. cupida</i> | 1 | 0.56318 | 0.56318 | 16.405 | < 0.001 |
| Light | 1 | 2.32047 | 2.32047 | 67.593 | < 0.001 |
| <i>Baetis</i> x <i>L. cupida</i> | 1 | 0.27615 | 0.27615 | 8.044 | < 0.01 |
| <i>Baetis</i> x Light | 1 | 1.02115 | 1.02115 | 29.745 | < 0.001 |
| <i>L. cupida</i> x Light | 1 | 0.22675 | 0.22675 | 6.605 | < 0.025 |
| Error | 31 | 1.06420 | 0.03433 | | |
| Total | 63 | 6.44787 | 0 | | |

After being touched by the antenna of a stonefly, *Baetis* nymphs frequently swam away.

DISCUSSION

It has been established that live aquatic insect larvae readily return to the stream bed in daylight hours (Luedtke and Brusven 1976, Walton 1978, Ciborowski and Corkum *this volume*). Our investigation demonstrated that small *Baetis* nymphs settled more slowly onto the stream bed than did larger nymphs of the same species. Mature *Baetis* and *L. cupida* nymphs settled so quickly that it was not possible to detect any substrate influence with the data collecting procedures that we used. Small nymphs settled more quickly when food was present on the stream bed. With the exception of immature *Baetis* settling onto bare substrates, our study and that of Walton's (1978) indicate that a stonefly predator had no influence on the settling behaviour of mayflies.

Our study clearly shows that tactile invertebrate predators such as *I. elongatus* can influence the drift of two species of mature herbivorous mayflies. The swimming form, *Baetis*, drifted in significantly greater numbers than the crawling form, *L. cupida*. *Leptophlebia* evidently prefers substrates with vegetation for cover. Our experiments also showed that the stonefly predator had no effect on immature *Baetis*. It is not known what influence stonefly predators would have if larger numbers of small *Baetis* had occupied the stream bed. Larger prey, however, are more likely to be detected by foraging predators and also provide a greater return per search than smaller prey (Siegfried and Knight 1976). Siegfried and Knight (1976) showed that the stonefly predator *Acroneuria californica* Banks selected large size classes of both chironomid larvae and baetid nymphs from the Squirrel River, California.

Table 4. Nymphal measurements (head capsule width and total body length) for representative specimens used in the autumn, 1977 and spring, 1978 laboratory experiments. (Values are in mm).

| Species | Date | n | H.C.W. $\bar{x} \pm \text{s.d.}$ | T.B.L. (cerci & antennae excluded) $\bar{x} \pm \text{s.d.}$ |
|---------------------|-----------------|----|-------------------------------------|--|
| <i>Baetis</i> sp. | Oct. 1977 | 50 | 0.545 \pm 0.054 | 2.713 \pm 0.430 |
| <i>I. elongatus</i> | " | 50 | 2.523 \pm 0.342 | 12.550 \pm 2.221 |
| <i>Baetis</i> sp. | Apr. - May 1978 | 50 | 0.967 \pm 0.092 | 5.655 \pm 0.804 |
| <i>L. cupida</i> | " | 50 | 1.812 \pm 0.287 | 8.535 \pm 1.644 |
| <i>I. elongatus</i> | " | 50 | 3.157 \pm 0.321 | 18.760 \pm 2.389 |

Baetis and *L. cupida* nymphs may respond differently to the presence of each other than they do to predators. No increase in drift was observed for either herbivore when the second mayfly species was present even though the nymphal numbers on the stream bottom were doubled. It is likely that a large actively foraging carnivore having long antennae and cerci would encounter mayflies more often than either mayfly species would encounter each other. In drift-density studies of a single herbivorous species, it is often assumed, though not always stated, that less abundant cohabiting species do not influence the drift of the species being investigated. Our study shows that this assumption can be accepted even when the cohabiting herbivores are equal in density.

There have been relatively few studies investigating the influence of riverine insect predators on the behaviour of prey species. Walton (1978) showed that the stonefly *Acroneuria abnormis* (Newman) had no effect on the drift of the mayfly *Stenonema interpunctatum* (Say). This mayfly, like other heptageniids, typically clings to the undersides of rocks and therefore would not be expected to avoid benthic predators by entering the water column. In another study, Corkum (unpublished data) found that the stonefly predator *Paragnetina media* (Walker) significantly influenced the drift of mature *Baetis vagans* McDunnough at night. Hildrew and Townsend (1977) showed that differences in predation rates of the caddisfly *Plectrocnemia conspersa* (Curtis) corresponded to differences in behavioural responses of the prey once prey entered the predator's net.

Predation is probably the greatest factor accounting for mortality of benthic insect populations (Benke 1978). Rheophilic mayflies are vulnerable to both visual and tactile predators. Visual predators (i.e. fish) are probably more successful during the day and capture insects primarily within the water column, whereas tactile predators hunting on the substrate are effective both day and night. Immature baetids present within the water column are unlikely to be detected by visual predators because of the small size and transparency of these mayflies (Allan 1978). Thus, immature baetids may drift at any time to avoid benthic tactile predators and not risk capture by visual predators. Mature drifting mayflies, however, could be highly vulnerable during the day and thus may use drift avoidance behaviour to tactile predators only at night. Charnov *et al.* (1976) showed that 80% of baetid nymphs occupied dark corners of an aquarium whenever a fish predator, *Oncorhynchus nerka*, was introduced, compared to only 20% occupying dark corners when the predator was absent. The *Baetis* sp. of our experiments were similar to *B. vagans* in their thoracic and abdominal pigmentation. The lateral view of these mature nymphs appears to mimic the eyes of a much larger invertebrate. This pigmentation pattern may in part protect *Baetis* from visual benthic predators. Nevertheless, it is evidently a greater risk for mature *Baetis* to enter the water column during daylight hours than to remain on the stream bed.

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RESUME

Des expériences ont été effectuées au laboratoire avec différents types de substrat pour étudier les déplacements de deux éphéméroptères herbivores et d'une plécoptère carnivore. La lumière, l'unique facteur étudié, provoqua le déplacement de jeunes larves de l'espèce *Baëtis*. Le prédateur appelé *Isogenoides elongatus* (Hagen) agit puissamment sur les larves de l'espèce *Baëtis* et de l'espèce *Leptophlebia cupida* (Say) qui ont atteint le stade de maturité. En présence de végétations artificielles, les larves de l'espèce *L. cupida* se déplacèrent en nombre beaucoup moins considérable. La présence de larves de *Leptophlebia* agit fortement sur le déplacement de représentants de l'espèce *I. elongatus* en pleine obscurité. Les deux espèces herbivores associées ne jouent aucun rôle sur le déplacement d'une ou l'autre. Il semble qu'au cours de la journée, les larves de l'espèce *Baëtis* au stade de maturité courent un plus grand risque d'être la proie de prédateurs si elles s'aventurent dans la colonne d'eau que si elles restent sur le fond du cours d'eau.

ZUSSAMENFASSUNG

Laboratoriumsexperimente wurden durchgeführt, um die Drift Reaktionen zweier pflanzenfressender Eintagsfliegen und einer fleischfressenden Steinfliege mit verschiedenen Substrat-Typen zu studieren. Licht war der einzige untersuchte Faktor, der die Drift unreifer *Baëtis* sp. Nymphen beeinflusste. Der Preadator, *Isogenoides elongatus* (Hagen), hatte entscheidenden Einfluß auf die Drift der beiden reifen *Baëtis* sp. und *Leptophlebia cupida* (Say) Nymphen. Bei künstlicher Vegetation drifteten die *L. cupida* Nymphen in viel geringerer Zahl. Die Gegenwart von *Leptophlebia* Nymphen hatte großen Einfluß auf die Drift von *I. elongatus* Exemplaren während der Dunkelperioden. Die beiden ko-habituierenden, pflanzenfressenden Arten zeigten keine Drift Reaktion zueinander. Man vermutet, daß bei Tageslicht für reife *Baëtis* Nymphen eine größere Gefahr der Praedation besteht, wenn sie sich in die Wassersäule begeben, als wenn sie im Flußbett bleiben.

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