Balancing risks? Responses and non-responses of mayfly larvae to fish and stonefly predators

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Summary. In a series of laboratory experiments we examined the hypothesis that larvae of stream mayflies would respond to the presence of two different types of predators in such a way as to minimize their risk of being consumed by each. Positioning of larvae (whether they frequent the top, sides, or bottom of stones) of *Baetis tricaudatus* and *Ephemerella subvaria* was altered by the presence of predaceous stoneflies (*Agnetina capitata*) with a larger proportion of the population occurring on the upper surfaces, where the probability of encountering the predator was lowest. The presence of a benthivorous fish (*Cottus bairdi*) had no significant effects on positioning of the mayfly larvae. Lack of fish effects may reflect an inability of the mayflies to detect or respond to sculpins, or alternately may indicate that sculpins do not normally present a important predation risk for these mayflies. Failure of mayfly prey to account for fish predators when responding to the presence of stoneflies appears to explain facilitation previously observed between stoneflies and sculpins.

Key words: Positioning – Relative risk of predation – Facilitation – Predator avoidance – *Ephemerella* – *Baetis* – *Cottus* – *Agnetina*

Behavioral responses to the presence of a potential predator are widely reported among a wide array of aquatic animals (e.g. Dill 1987; Sih 1987). However, organisms in natural environments usually face a number of different types of predators, and any one type of response is unlikely to be effective for all types of predators. In fact, responding to one type of predator may increase risk from other types of predators. For example, Wilson (1971) reports several studies that describe how predaceous flies and birds exploit insects fleeing from the foraging activities of army ant columns.

In headwater streams prey species such as mayfly larvae face predation from two very different groups of predators, vertebrate and invertebrate. Most of the time invertebrate predators such as stoneflies will remain on the underside of stones, especially in the presence of fish (Soluk and Collins 1988a). Vertebrate predators such as fish are usually unable to access the underside of stones, and can only capture prey on the tops and sides of stones. Prey therefore face a dilemma when they position themselves on stones,

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if they move off the underside of stones to minimize risk of predation from invertebrate predators then they increase their risk from fish and vice versa. How this positioning dilemma is resolved may give us insight into what prey experience as relative risk from these different types of predators.

We use the term "positioning" here to refer to how invertebrates use the surfaces (top, sides, or bottom) of individual stones. There is little quantitative information on positioning of stream invertebrates and how this is affected by the presence of predators, however, diel shifts in the positioning of mayfly larvae and other prey species on stones have generally been attributed to avoidance of fish predators (e.g. Elliot 1968; Peckarsky 1984; Kohler 1985; Allan et al. 1986).

Although there is little evidence of positioning responses, stream invertebrates are known to exhibit a variety of other responses to the presence of fish and invertebrate predators. Responses of mayfly larvae to the presence of stonefly predators has been extensively examined (e.g. Peckarsky 1980; Williams 1987) and appears to depend on short range chemical and tactile cues. General responses to the presence of fish, such as changes in patterns of movement or substrate selection, have been reported for a number of different types of stream invertebrates including mayflies (Charnov et al. 1976), stoneflies (Feltmate et al. 1986; Williams 1986; Soluk and Collins 1988a) and amphipods (Williams and Moore 1985; Andersson et al. 1986).

Such responses indicate that prey species such as mayfly larvae can detect predators, however, they provide little insight into whether positioning on the substrate is affected by predators and what consequences this may have both for prey populations, and for how invertebrate and vertebrate predators interact in streams. In this study we examine the positioning responses of two common types of Ephemeroptera prey to the presence of a predaceous stonefly and a benthivorous fish. We than use this data in an attempt to evaluate the relative importance of these predators and predict possible consequences for prey that must coexist with both types of predators simultaneously.

Methods

Positioning responses of larvae of the mayflies *Baetis tricaudatus* and *Ephemerella subvaria* to the presence of the perlid stonefly *Agnetina capitata* and the sculpin *Cottus bairdi* were studied in a recirculating laboratory stream (see Soluk

and Collins 1988 b) with a mean current velocity in the working area of 20 cm/s and a water temperature of 13° C. Sculpins were used in preference to other stream fish (e.g. trout) since they are largely benthivorous and have been cited as being more likely to have a direct impact on benthic invertebrate populations (see Allan 1983).

A concrete brick (19.0 × 9.2 × 5.8 cm) was used in preference to a natural stone since it provided three well defined surfaces (top, sides, and bottom). The brick was supported off the bottom of the stream by four spacers (3 mm diameter, 7 mm high) which allowed mayfly and stonefly larvae to access the bottom but excluded the sculpin.

Positioning of each species of mayfly was determined in separate replicated experiments conducted under both light and dark conditions. For each replicate two stream tanks were used. Mayfly larvae (either *Baetis* or *Ephemerella*) were introduced into each stream and their position (top, sides, or bottom) on the brick was recorded after one hour. The predator (either two larvae of the stonefly *Agnetina capitata* or one sculpin *Cottus bairdi*) was then introduced into one of the streams and after one hour the position of mayfly larvae were again recorded in both streams. Predator treatment and control streams were alternated for each experimental replicate. Number of larvae introduced into each stream tank in each replicate was 75 for *Baetis* and 30 for *Ephemerella*. *Baetis* larvae drifted much more frequently than *Ephemerella*, thus a higher initial density of this species was required to ensure that sufficient numbers of larvae were on the substrate during the experiment.

An interval of 1 h exposure to predators was chosen in all initial experiments to minimize any complicating effects of reduction in prey number due to predation. Positioning in control tanks did not change substantially over 1,2, or 4 h intervals for either *Baetis* or *Ephemerella*. In experiments carried out in the dark, mayfly larvae were kept in darkness for at least one hour before being introduced into the stream tanks. Positioning in the dark was observed with the aid of a small flashlight with a deep red filter.

An additional experiment was run to determine whether the apparent lack of responses by *Ephemerella* to the presence of sculpins was a function of the duration of the original experiments. Design was the same as those above except that the sculpin's mouth was sewn shut with two stitches of thread, and the *Ephemerella* larvae were in the presence of the sculpin for a 24 h (12 h light, 12 h dark) period.

All statistical comparisons were made using a Mann-Whitney test (Zar 1984), since some of the data exhibited significant departures from a normal distribution (normality test, Shapiro and Wilk 1965).

Results

Median total number of *Baetis tricaudatus* larvae on the brick was significantly reduced by the addition of stoneflies from 53.5 to 41.5 larvae/brick ($P=0.037$) in the light, and from 50.0 to 35.0 larvae/brick in the dark ($P=0.002$). This change in total number on the brick necessitated use of proportional data to assess effects of *Agnetina* on positioning. *Baetis* exhibited significant changes in positioning in the presence of *Agnetina*. Proportion of *Baetis* larvae on the top of the brick was significantly increased in the presence of the stoneflies (Fig. 1) both in the light ($P=0.020$)
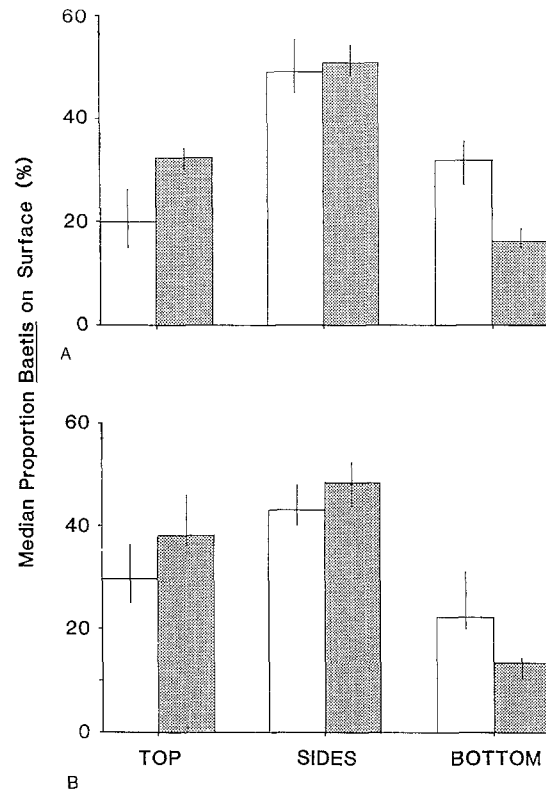

Fig. 1. Median proportion of *Baetis tricaudatus* larvae on the various surfaces of the brick in the light ($N=6$) (**A**) and dark ($N=7$) (**B**), in the presence (▦) and absence (□) of the stonefly *Agnetina*. Vertical bars represent the upper and lower quartiles. "N" is the number of replicates, each involving 75 *Baetis* larvae

and in the dark ($P=0.015$), while proportion on the underside (Fig. 1) significantly decreased in both light ($P=0.031$) and dark ($P=0.002$) conditions. Proportion of *Baetis* larvae on the sides of the brick was not significantly affected in either light ($P=1.00$) or dark ($P=0.20$) by the presence of *Agnetina*. Mean number of *Baetis* consumed by *Agnetina* over both light and dark experiments was 4.42 larvae/stream.

Total number of *Ephemerella subvaria* larvae on the brick was not significantly affected by the presence of stonefly larvae in the light (medians of 28.5 larvae/brick without and 28.5 larvae/brick with stoneflies, $P=0.51$). However, in the dark there was a small but significant increase in the number of *Ephemerella* larvae on the brick (medians of 27.0 larvae/brick without and 30.0 larvae/brick with stoneflies, $P=0.023$) when *Agnetina* was added. *Ephemerella* larvae exhibited no significant positioning response in the light (Fig. 2) (top $P=0.75$, sides $P=0.47$, bottom $P=0.34$), however, proportion of *Ephemerella* larvae on the underside of the brick was significantly reduced in the dark ($P=0.012$) by the presence of *Agenetina*. Under dark conditions, there were no significant changes in the proportion of larvae on either the tops ($P=0.61$) or sides ($P=0.20$) of the brick when these surfaces were considered separately. However, when proportion of *Ephemerella* larvae on the top and sides were pooled (to yield a value for all upper surfaces of the brick), a significant increase ($P=0.012$) was found in response to the presence of *Agnetina*. Similar pool-
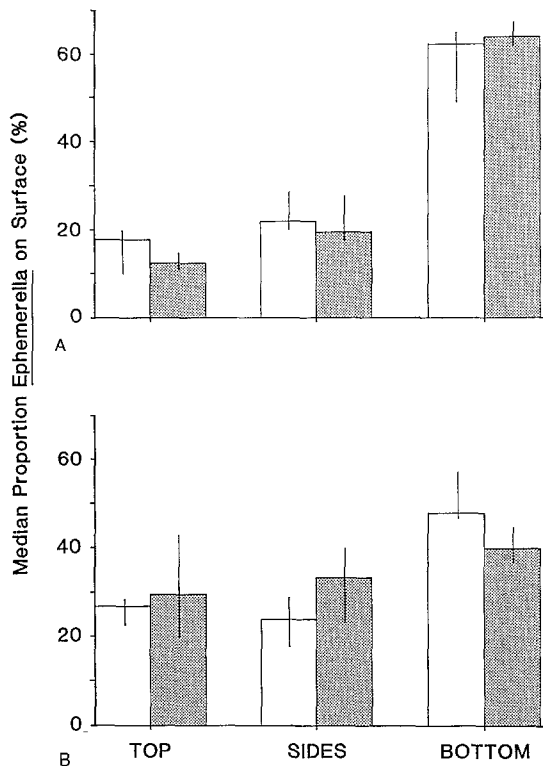
Fig. 2. Median proportion of *Ephemerella subvaria* larvae on the various surfaces of the brick in the light ($N=6$) (A) and dark ($N=7$) (B), in the presence (▨) and absence (□) of the stonefly *Agnetina*. Vertical bars represent the upper and lower quartiles. "N" is the number of replicates, each involving 30 *Ephemerella* larvae
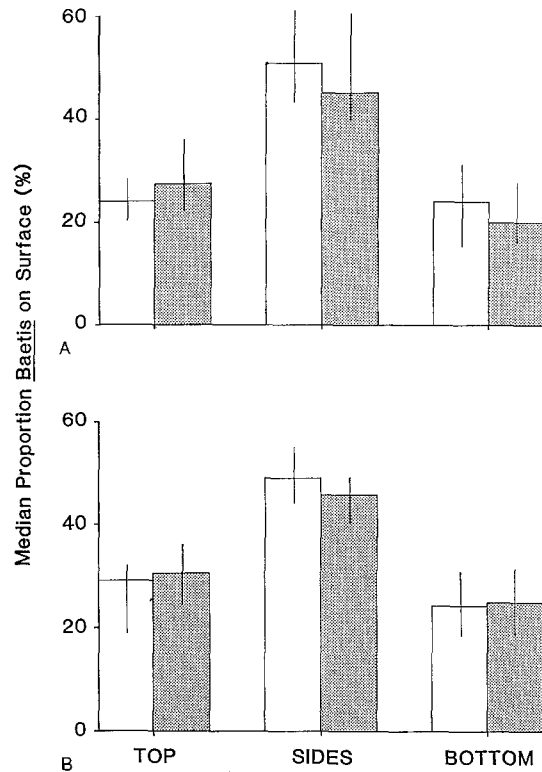

Fig. 3. Median proportion of *Baetis tricaudatus* larvae on the various surfaces of the brick in the light ($N=11$) (A) and dark ($N=7$) (B), in the presence (▨) and absence (□) of a sculpin (*Cottus bairdi*). Vertical bars represent the upper and lower quartiles. "N" is the number of replicates, each involving 75 *Baetis* larvae

ing of data for top and sides under lighted conditions, indicated no significant effect ($P=0.34$) of *Agnetina* on positioning of *Ephemerella* larvae. Mean number of *Ephemerella* larvae consumed by *Agnetina* during the experiments was 1.41 larvae/stream.

The presence of sculpins had no significant effect on the positioning of *Baetis* larvae. Proportion of larvae on the various surfaces of the brick (Fig. 3) was not significant changed by the presence of sculpins in the light (top $P=0.22$, sides $P=0.87$, bottom $P=0.51$) or in the dark (top $P=0.30$, sides $P=0.30$, bottom $P=0.89$). Pooling top and sides yielded similar results in both light ($P=0.39$) and dark ($P=0.90$). Presence of sculpins also had no significant effect on the total number of *Baetis* larvae on the brick in either light ($P=0.53$) or dark ($P=0.90$) conditions. Mean number of *Baetis* consumed by sculpins during these experiments was 3.5 larvae/stream.

Ephemerella larvae were also remarkably non-responsive to the presence of sculpins. There was no significant effect of sculpins on the proportion of larvae on the top ($P=0.79$), sides ($P=0.79$), pooled top and sides ($P=0.72$), or bottom ($P=0.72$) of the brick under dark conditions (Fig. 4). In the ligth there were also no significant effects of sculpins on the proportion of *Ephemerella* larvae on the top ($P=0.94$), sides ($P=0.61$), pooled top and sides ($P=0.70$), or bottom ($P=0.70$) of the brick (number of replicates $=7$). In addition, there was no significant effect ($P=1.00$) on total numbers *Ephemerella* on the brick. Mean number of *Ephemerella* larvae consumed by sculpins was 4.33 larvae/stream.
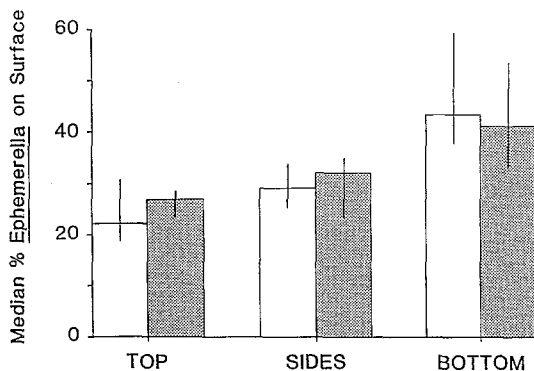
Fig. 4. Median proportion of *Ephemerella subvaria* larvae on the various surfaces of the brick in the dark ($N=9$) in the presence (▨) and absence (□) of a sculpin (*Cottus bairdi*). Vertical bars represent the upper and lower quartiles. "N" is the number of replicates, each involving 30 *Ephemerella* larvae

Even after 24 h (12 h light, 12 h dark) in the presence of a sculpin, there were no significant effects on proportions of *Ephemerella* larvae on the top ($P=0.81$), sides ($P=0.23$), pooled top and sides ($P=0.56$), or bottom ($P=0.56$) of the brick (Fig. 5).

Discussion

Soluk and Collins (1988a) examined positioning by *Agnetina* larvae and found that these stoneflies normally spent more than 95% of their time on the underside of the sub-
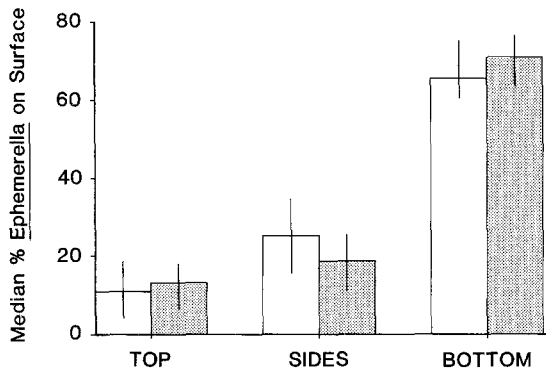
Fig. 5. Median proportion of *Ephemerella subvaria* larvae on the various surfaces of the brick after 24 h (12 h dark, 12 h light) ($N =$ 6), in the presence (▨) and absence (□) of a sculpin (*Cottus bairdi*). Vertical bars represent the upper and lower quartiles. "N" is the number of replicates, each involving 30 *Ephemerella* larvae

strate. Thus the responses to *Agnetina* larvae exhibited by both *Baetis* and *Ephemerella* larvae seem prudent, in that they moved away from the substrate surface (i.e. the underside of the brick) where they were most likely to encounter this predator.

Avoidance of the general vicinity of stonefly larvae has previously been observed for *Baetis* which usually swims away (Peckarsky 1984; Williams 1987). This type of behavioral response probably accounts for the reduced abundance of *Baetis* on the brick since larvae swim off it, entering the drift or clinging on to the edges of the plexiglas in the stream. In contrast to *Baetis*, *Ephemerella* larvae usually respond by crawling away from the vicinity of a stonefly (Williams 1987). At any particular time almost all *Ephemerella* larvae were on the brick. The slight but statistically significant increase in total number of larvae on the brick may reflect an increased tendency of *Ephemerella* larvae to hold tightly to the substrate in the presence of *Agnetina*. Examination of video tapes of stoneflies feeding on *Ephemerella* (D.A. Soluk, unpublished data), suggests that holding tightly to the substrate may be a way to resist attacks by stoneflies which often give up on larvae they cannot dislodge.

The positioning responses of *Baetis* and *Ephemerella* also differed in that *Ephemerella* moved to the upper surfaces only in the dark, whereas *Baetis* responded to the presence of stoneflies in both dark and light conditions. This could reflect some fixed behavior pattern of *Ephemerella* larvae (i.e. not to move much, or not to move from the bottom under lighted conditions) which reduces their susceptibility to diurnally active visual predators such as fish, or may simply be a consequence of their being less sensitive than *Baetis* to stoneflies that are largely inactive under lighted conditions.

Mayfly larvae are favoured food items of many stream fish, thus the lack of response by *Ephemerella* and *Baetis* to the presence of this type of predator was unexpected. Why this apparent lack of response to the presence of a potentially important benthic fish predator? There are three possible explanations for this lack of response: 1) some undefined inadequacy in the design of experiments which interfered with the ability of the mayflies to detect sculpins; 2) mayflies cannot detect the presence of sculpins; or 3) mayfly larvae detect, but have no positioning response to the presence of sculpins.

It might be argued that detection of the presence of fish was not possible because of some problem with the design of the experiments. However, under similar experimental conditions larvae of the stonefly *Agnetina* were clearly able to detect and respond to the presence of sculpins (Soluk and Collins 1988a). It therefore seems likely that mayfly larvae either are unable to detect the presence of sculpins or at least that they do not exhibit a positioning response to the presence of these predators.

Difficulty in detecting some types of fish predators may be a real constraint for mayfly larvae. Sculpins exhibit a type of foraging that consists of short periods of movement, a variable period of scanning for prey, followed by another period of movement. This type of foraging lies somewhere in the continuum between a sit-and-wait and an free-ranging strategy. Such a foraging strategy may make sculpins more difficult to detect than a more active fish (e.g. trout), unless the potential prey has a means of detecting them via long range chemical cues.

Perhaps the simplest explanation for the lack of response by mayfly larvae is that predation risk from sculpins, or perhaps fish in general, may be so low that there has been no selective pressure to develop positional responses to this type of predator. This view is consistent with suggestions that fish effects and biotic effects in general may be frequently overwhelmed by the effects of stochastic abiotic disturbances in some streams (see Peckarsky 1983). However, attributing the lack of responsiveness to the absence of strong biotic interactions in stream communities does not explain the responsiveness of mayfly larvae to predatory stoneflies or explain why stoneflies themselves are known to exhibit clear responses to the presence of predaceous fish (Williams 1986; Soluk and Collins 1988a).

Mayflies may not exhibit a positioning response to sculpins because inidivudals may have only a low probability of encountering such predators. Little is known about actual encounter rates between fish and mayfly larvae or between fish and stoneflies or other invertebrates in streams. Ware (1973) evaluated some of the components of risk for epibenthic prey exposed to the presence of trout, and found that size was one of the most important factors in determining the distance at which fish could perceive prey. The relatively large size (> 15 mm) of many stoneflies and crustaceans means that they are probably more readily detected over a much wider radius by a foraging fish, and this may effectively increase the encounter rate between these animals and fish to such an extent that some behavioral avoidance mechanism is necessary. Mayflies by virtue of their small size may be difficult to detect, and thus they would have relatively low encounter rates with benthivorous fish and experience corresponding low risk to this type of predator.

In addition to their small size, mayflies such as *Baetis* and *Ephemerella* have relatively short lives compared to many predaceous stoneflies which can have generation times of 2 or 3 years. Even if mayflies and stoneflies have a similar number of encounters with fish per unit time, the short larval period of many mayflies would result in lower risk over the lives of individual mayfly larvae when compared with that experienced by stoneflies.

Responding to one type of predator and not another appears to have serious negative consequences for individual *Ephemerella* larvae. Soluk and Collins (1988b) found that when *Agnetina* larvae were present, sculpins almost

doubled the rate at which they were able to capture *Ephemerella* in laboratory stream microcosms. Our finding that the presence of a stonefly causes *Ephemerella* larvae to move to the upper surfaces of stones explains this facilitation between sculpins and stoneflies, since such a behavioral response leads to increased risk of predation when sculpins are present.

Baetis larvae show a pattern of responses and non-responses to predators similar to that observed for *Ephemerella*, however, Soluk and Collins (1988b) found no evidence of facilitation between stoneflies and sculpins with respect to *Baetis*. This apparent contradiction may be explained by differences in the type of predator avoidance behavior used by these two types of mayflies. In the presence of *Agnetina*, *Baetis* larvae tended to leave the substrate, clinging to edges of plexiglas or drifting, whereas *Ephemerella* larvae usually just crawled away. Our observation of the feeding behavior of sculpins suggests that they do not capture many mayflies from the water column, but feed primarily on individuals that are attached to substrate. Thus although proportion of *Baetis* larvae off the bottom of the substrate is decreased by the presence of *Agnetina*, any benefit for the sculpin is minimized because many *Baetis* larvae are entering the drift and becoming unavailable. For fish such as trout or dace that are more effective at feeding on the drift, indirect facilitation by stoneflies might be found for *Baetis* and not for *Ephemerella*.

There is little evidence of strong effects of fish on benthic invertebrate communities in streams from removal or enclosure/exclosure experiments (Allan 1983; Flecker and Allan 1984; Reice and Edwards 1986). Allan (1983) has suggested that this may be because benthic invertebrate communities are well adapted to deal with the presence of fish predators that are almost never predictably absent from streams. While fish in general are seldom absent, species composition and abundances can vary widely even within a single reach (Grossman et al. 1982), thus actual risk from fish predators may vary widely. The "well adapted" hypothesis of Allan predicts that stream invertebrates should in general have a variety of effective morphological or behavioral adaptations to avoid fish. Our study does little to support the view that mayflies are well adapted at coping with the presence of predaceous fish, at least from a behavioral perspective.

The lack of reactive positioning responses by mayflies to risk from sculpins cannot in itself be seen to support the contention that fish predation is not an important factor affecting populations of stream invertebrates. These animals may have a variety of fixed behaviors, or at least more subtle reactive responses that reduce their susceptibility to fish. However, our data suggests that on a relative scale, risk from invertebrate predators is a more important factor in affecting behavior and positioning of mayfly larvae.

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