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Upstream resource abundance determines the food searching behavior of a stream grazer: Effect of microalgal cues

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Abstract

To maximize their growth, grazers should shift their behavior to balance the cues from predators and periphyton in the field. As a stream grazer seeks periphyton that are heterogeneously distributed, a shift in its behavior on the basis of the intensity of microalgal cues would be beneficial as it would allow the grazer to feed on more periphyton. Using a caddisfly grazer, *Glossosoma* larvae, we conducted a laboratory channel experiment with upstream experimental plates having four levels of periphyton abundance (control, low, medium, and high), and we recorded the movement behavior of the larvae. As periphyton abundance increased, both larval crawling time and the total crawling distance to the periphyton patch significantly decreased and the directness of the crawling path significantly increased. That is, larvae crawled in various directions as they approached a relatively low-abundance periphyton patch, whereas they crawled straight to the high-abundance periphyton patch in a short time. The behavior change was likely due to the detection of some microalgal cue, which should intensify with an increase in upstream periphyton abundance.

Chemical and visual cues play important roles in the interactions between organisms (Lima 1998; Burks and Lodge 2002). Many studies have established that these cues are involved in predator–prey interactions. By recognizing predator cues, prey can change their life histories, morphologies, activity, and habitat use to avoid predation (McIntosh et al. 1999; Miyasaka et al. 2003; Turner and Montgomery 2003). Likewise, predators can be attracted to food patches by recognizing cues from animal prey (Brönmark and Hansson 2000; Burks and Lodge 2002). Communication via cues also occurs between predators (herbivores) and primary producers in aquatic ecosystems. Numerous studies have shown morphological changes of primary producers on the basis of predator cues in aquatic ecosystems (Brönmark and Hansson 2000), whereas fewer studies have examined herbivore responses to producer cues, and these studies were limited to consumption (DeMott 1986) and habitat use (Doi et al. 2006). Although the responses to such cues would require behavioral changes by the herbivore, it remains unclear whether herbivores perform searching behavior in response to such cues (e.g., changes in movement path; Bowne and White 2004).

In stream ecosystems, grazers respond to a range of heterogeneous periphyton resources because periphyton abundance is patchily distributed (Biggs 1996). Various grazers perform area-restricted searches to obtain abundant food resources (Hart 1981; Kohler 1984). Caddisfly and mayfly grazers have alternative behaviors depending on periphyton abundance; they move quickly until arriving at an abundant periphyton patch, and then they move slowly within the patch (extensive and intensive searches, respectively; Kohler 1984; Poff and Ward 1992; Katano et al. 2005*a*). On the basis of the hypothesis that cues from periphyton (i.e., microalgal cues) mediate communication between grazers and periphyton (Doi et al. 2006), we predict that the behavior of grazers would change on the basis of periphyton cues, which would initiate grazers to search for periphyton patches; and the behavior would change according to the intensity of the cue (i.e., abundance of food source).

In the present study, we tested the above hypothesis regarding communication between periphyton and grazers using a caddisfly grazer, *Glossosoma* sp. (Trichoptera: Glossosomatidae). We performed a laboratory channel experiment to investigate whether *Glossosoma* larvae changed their extensive search behavior (before arriving at a periphyton patch) according to the abundance of an upstream periphyton patch. The genus *Glossosoma* is a well-known grazer that feeds on periphyton. The larvae crawl on the surfaces of stones in riffles with dome-shaped sand cases, and are often the dominant grazer in Japanese streams with hard substrates (Doi and Katano 2008). Doi et al. (2006) reported that *Glossosoma* larvae increased their habitat use according to an increase in periphyton abundance, indicating that they may be detecting and using a microalgal cue.

Methods

We cultivated periphyton mats on clay plates from January to March 2004 in a riffle of the Shigo-gawa Stream, a tributary of the Kino-kawa River, Nara Prefecture, Japan (34°22′66″N, 136°01′00″E). We prepared four types of substrate plates (10 × 10 × 0.3 cm): untreated clay plates (control), low-abundance periphyton plates

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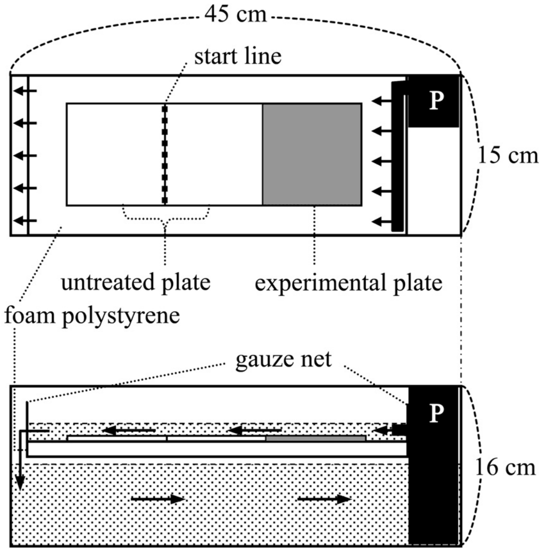

Fig. 1. (A) Top and (B) side views of the experimental channel. Experimental plates had various periphyton abundances. Arrows and P indicate the direction of water flow and the water pump, respectively.

(cultivated for 10 d in the stream), medium-abundance periphyton plates (32 d), and high-abundance periphyton plates (67 d). The plates were set at approximately 10 cm above the bottom of the stream to reduce grazing.

A recirculating experimental channel (45-cm length, 15-cm width, 1.5- to 2.0-cm water depth on experimental zone, and 8- to 9-cm water depth on water exchange zone) was used for the experiment (Fig. 1). Stream water, which was drawn from the Shigo-gawa Stream and filtered with 0.25-mm mesh sieve immediately before the experiment, was used to fill the channel, and the water was exchanged every trial. Current velocity was maintained at 15.0 ± 3.9 cm s^{-1} (mean ± SE, $n = 20$) at the start line (*see* below) with an aquarium water pump (E-roka PF-200; GEX); this velocity approximated the velocity where the test larvae were collected. The current velocity was measured with a portable current meter (model CW-7WP, Cosmo Riken). To avoid increases in water temperature, the experimental channel was placed in a light and cold room (air temperature 4.0°C) under seminatural light conditions (light intensity approximately 5000 lux), and water temperature was kept around 5.0–7.5°C, which ranged within diurnal fluctuation of the Shigo-gawa Stream water in March.

Just before the experiments, we collected *Glossosoma* larvae from the Shigo-gawa Stream (current velocity at the collecting points: 19.3 ± 2.6 cm s^{-1}) and transported them to the laboratory in a cooler box. In the center of the experimental zone, two untreated plates and one experimental plate (control, low-, medium-, or high-abundance plate) were arranged in contact with each other (Fig. 1A). The experimental plate was placed upstream, toward the water pump. The pump was started 5 min before each trial to circulate the microalgal cue in the experimental channel. At the beginning of each experiment, we randomly selected one *Glossosoma* larva (2.2 ± 1.1 mg in dry weight, fifth instars) and arranged it along the start line between the two untreated plates. We recorded the movement of each larva for up to 30 min after the start of the experiment using a video camera (Handycam DCR-PC350, Sony) with a tripod, which was set about 1 m over the channel. Five trials were performed for each of the four types of periphyton plates, each with a naive larva, and trials were randomly replicated from 09:00 h to 15:00 h.

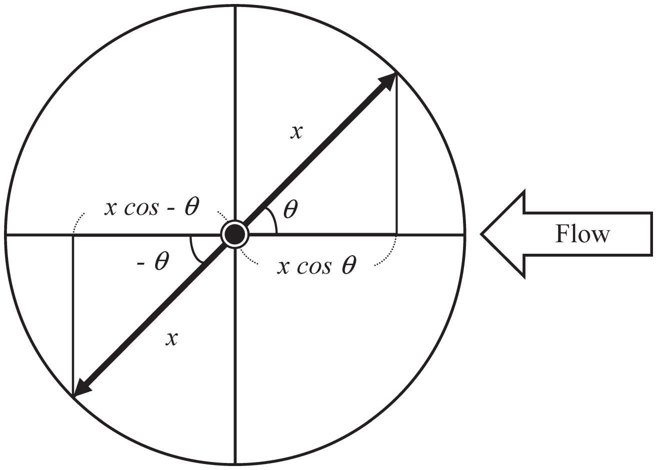

Fig. 2. Schematic illustration of calculation of the crawling distance and degree of directness. The closed circle and the arrowheads show the initial position and the position of the larva 3 min later. The crawling distance corresponds to the length of the arrow, and the directness was calculated according to Eq. 1.

We determined three larval behavior parameters: crawling time, total crawling distance, and degree of directness of the extensive search path. The crawling time was calculated as the period from start to finish (i.e., arrival at the experimental plate). To calculate the total crawling distance and degree of directness, we recorded the location of each larva every 3 min, so that in total about 10 locations per larva were recorded. The degree of directness, $S(t)$, was calculated using the following equation:

$$S(t) = x \cdot \cos \cdot \theta / x \qquad (1)$$

where x is the linear distance from the location at t min from the location at $t - 3$ min, and θ is the angle between the movement direction and the channel length (Fig. 2). The directness value ranges from -1 to 1. The degree of directness was 1 when a larva crawled absolutely straight to the treatment plate, whereas the value was -1 or 0, respectively, when a larva crawled straight in the opposite direction of the experimental plate or when a larva crawled vertically to the experimental plate. We estimated the degree of directness during the experiment as the averaged value for each 3-min interval. The total crawling distance was obtained by summing x values from the start to the finish of the experiment.

After the experiments, the dominant algal species of each experimental plate was identified under a microscope, and the periphyton abundance was measured. Each plate was divided into four areas, and the periphyton from each area was brushed off and placed in a container with 250 mL of water ($n = 4$). Dominant algal species were checked from the well-homogenized periphyton sample by a semiquantitative determination of relative abundance, which ranked algae on the basis of their percentage contribution to sample biovolume. The ranks were as follows: >60%, 60–30%, <30–5%, and <5%. Rankings were done by visually estimating the percentage abundance of the living algal species within 10 fields under a microscope (×400 magnification). The species with >60% and 60–30% ranks were considered as dominant algal species to characterize the periphyton community on each plate. Then, each periphyton sample was divided into two subsamples, and the subsamples were separately filtered through two precombusted glass filters (GF/C, Whatman International, pore size 1.2 μm). One was used to measure ash-free dry mass (AFDM) and the other the chlorophyll *a* (Chl *a*) content of periphyton. The former filter was dried at 60°C for 24 h and weighed to determine the dry weight; then the filter was combusted at 500°C for 2 h using a muffle furnace and reweighed to determine the ash weight. The AFDM of the periphyton was calculated as the difference between the dry weight and the ash weight. The latter filter was cut into small pieces and placed into a vial containing 20 mL of 99.5% ethanol. After preservation of the subsample in a dark refrigerator at 4°C for 24 h, the extracted pigments were measured using a spectrophotometer (model MPS-2000, Shimadzu). We determined Chl *a* contents from these data according to Steinman and Lamberti (1996) and expressed it as milligrams of Chl *a* per unit area (mg m^{-2}).

The three larval behavior parameters (crawling time, total crawling distance, and degree of directness) were compared among $\log_{10}(x + 1)$-transformed periphyton abundance (AFDM) by simple regression analysis. In addition, we tested the differences in AFDM and Chl *a* among the treatments using analysis of variance (ANOVA) and Tukey multiple comparison test. Finally, we tested the correlation between AFDM and Chl *a* contents of periphyton to confirm that there were no effects of accumulated detritus on the periphyton mat. We performed the statistical analyses using R version 2.6.2 software (R Development Core Team).

Results

The AFDM and Chl *a* were significantly different among the resource levels (ANOVA and Tukey multiple comparison, both $p < 0.01$, Table 1), except between control and low treatments for AFDM, and a significant correlation between both was observed ($R = 0.87$, $n = 16$, $p < 0.0001$). The dominant algal species were also different among the plates incubated for different times: high-abundance plates were dominated by both filamentous blue-green algae and large diatoms, whereas low- and medium-abundance plates were dominated by diatoms only.

Simple regression analyses showed that the AFDM of periphyton was significantly negatively correlated with the total crawling time of *Glossosoma* larvae, but was significantly positively correlated with the degree of directness of the larvae ($R = 0.45$ and 0.64, $p < 0.05$ and 0.01, respectively, each $n = 20$, Fig. 3). Although the larvae generally crawled toward the direction of flow (upstream) in all treatments (i.e., positive degree of directness, Fig. 3), the directness of their paths increased as periphyton abundance on the plates increased. On the other hand, no significant relationship was found between AFDM of periphyton and the total crawling distance of the larvae ($R = 0.20$, $p = 0.45$, $n = 20$). Total crawling distance in the control treatment was remarkably longer than those for the other resource levels (Fig. 3).

Discussion

Our results showed that the *Glossosoma* larvae moved upstream regardless of the amount of periphyton (scarce or abundant), but the larvae tended to crawl in more varied directions as they approached a relatively scarce periphyton patch, whereas they crawled straight to the abundant periphyton patch in a short time. From the starting point the larvae were not able to assess directly the abundance of the far upstream periphyton patches, at least not visually, because the head opening of the case is located at the bottom (Hoffman et al. 2006). Also, other stream grazers, such as baetid mayflies, cannot locate food patches visually and they require mouthpart contact to search food patches (Kohler 1984). In addition, the strong correlation between AFDM and Chl *a* contents of the experimental periphyton excludes the possibility that something other than microalgae in periphyton (e.g., accumulated detritus) affects the cue to *Glossosoma* larvae. Thus, some nonvisual cue allows the larvae to recognize the size of the upstream periphyton patch.

Our study design had a limitation: the rheotactic movements of the larvae, which were found at all resource levels, could not be decoupled from the effects of microalgal cues. Many stream organisms have positive rheotactic responses (Poff and Ward 1992; Olden et al. 2004; Hoffman et al. 2006), and rheotactic crawling toward the direction of flow is a basic movement of *Glossosoma* larvae. In fact, the degree of directness for the control

Table 1. Characteristics of experimental periphyton plates. Data are shown as mean ± SD. Variables labeled with same letters in a column do not differ significantly by Tukey multiple comparison ($p > 0.05$).

	Incubation time (d)	AFDM (g m^{-2})	Chlorophyll *a* (mg m^{-2})	Dominant species
Control	0	0.05±0.01a	0.00±0.00a	–
Low	10	0.22±0.05a	0.10±0.04b	*Cocconeis placentula*, *Achnanthes* spp.
Medium	32	0.77±0.15b	0.61±0.05c	*Achnanthes* spp., *Cymbella* spp., *Synedra ulna*
High	67	2.02±0.49c	3.48±0.70d	*Synedra ulna*, *Homeothrix* sp., *Phormidium* sp.

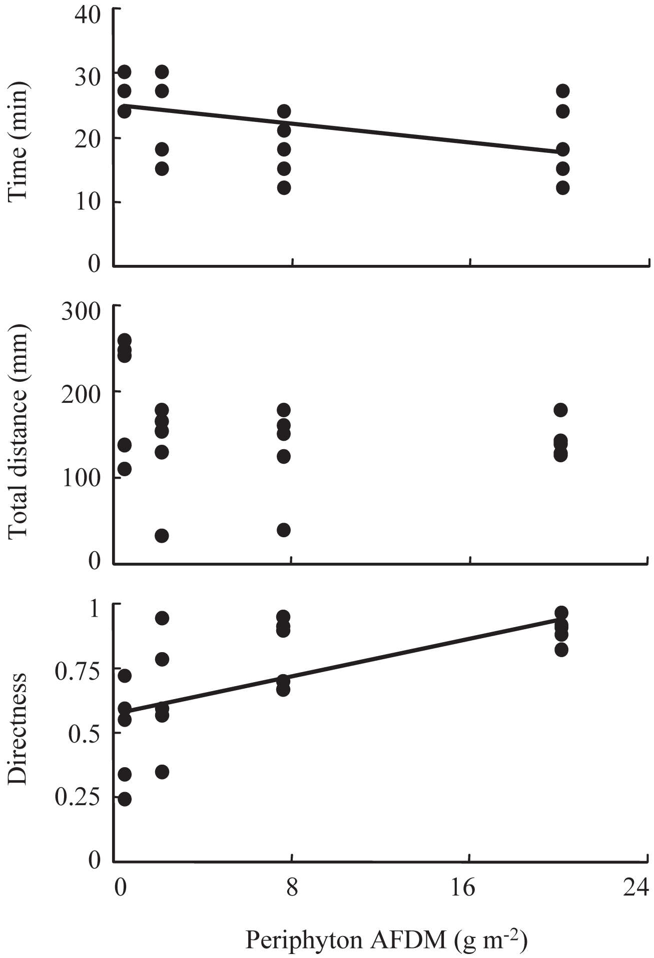

Fig. 3. Simple regression analysis between the crawling times, total crawling distances, and degree of directness of the *Glossosoma* larvae and periphyton abundance (AFDM). Within each graph, the statistically significant regression line is drawn ($p < 0.05$).

treatment was about half that for the high periphyton level. More intense upstream cues, however, would enhance the upstream movement of *Glossosoma* larvae. Therefore, our findings suggest that the *Glossosoma* larvae changed their searching behavior on the basis of the detection of some microalgal cue—one that increased with the increase in upstream periphyton abundance.

In this study, we estimated the effect of upstream resource abundance on a grazer's feeding behavior. In natural streams, however, turbulent currents are often observed (Allan and Castillo 2007). Dahl et al. (1998) suggested that shrimp can detect chemical cues from their back side and change their behavior. Thus, in natural streams grazers would likely respond to cues from various directions, but the responses under turbulent conditions to heterogeneous algal patches could not be estimated in this study. Additional laboratory and field studies are needed to fully understand the effect of the microalgal cues found in this study on grazers' behavior under natural conditions.

After they detected the microalgal cue, the larvae crawled in a short time straight to the abundant food patch. Because periphyton communities are patchily and heterogeneously distributed in the field (Biggs 1996), grazers perform extensive and intensive searches (i.e., movement to a new resource patch and an area-restricted search) to maximize feeding efficiency and the net rate of energy intake (Kohler 1984; Poff and Ward 1992). The heterogeneous distributions of various trichopteran grazers in the field are determined by microalgal abundance. The grazers respond to habitats with high food availability (Hart and Robinson 1990; Katano et al. 2005*b*, 2007), and an experimental plate with an abundant periphyton mat set in a stream was covered by trichopteran grazers within a few weeks (Lamberti and Resh 1983). The behavioral changes that we observed suggest a possible explanation for such a heterogeneous distribution of grazers in the field via the change in crawling behavior of the grazers from an area-restricted search to the straight movement on the basis of microalgal cues.

We did not identify the nature of the cue, that is, whether the microalgal cue was chemical, visual, or both. Some diatoms are known to drift from periphyton mats (Bothwell et al. 1989), and such drifting diatoms may have functioned as the microalgal cue for *Glossosoma* larvae. Diatoms drift more at night than during the daytime (Bothwell et al. 1989), and Doi et al. (2006) reported that the habitat use of abundant periphyton patches by *Glossosoma* larvae is greater at night than during the day. In addition, the different periphyton components among the experimental resource levels may change the microalgal cue. The periphyton mats in this study were dominated by diatom species, which are a good food resource for stream-grazing invertebrates (Allan and Castillo 2007). However, the abundant periphyton patches in natural streams are frequently composed of various algal taxa, such as diatoms, filamentous blue-green algae, and green algae (Biggs 1996). These different cues would likely have different effects on the behavior of grazers (c.f., multiple chemical cue to grazers in lakes, Burks and Lodge 2002). More research is necessary to identify the microalgal cue, which will provide a better understanding of the mechanisms of communication via cues between herbivores and primary producers.

Natural selection would foster the detection of such cues and consequent behavioral changes, because grazers that can detect microalgal cues could react by spending more time feeding, which would allow their growth performance to increase, especially under food limitation. Indeed, Feminella and Resh (1990) reported that low periphyton biomass reduced the growth and survival of trichopteran grazers. In addition, low periphyton availability resulted in an increased distance of *Glossosoma* larval movements, indicating that the larvae had to travel farther to maximize their food intake (Poff and Ward 1992), which increases energy costs and thus lowers fitness.

Although *Glossosoma* grazers are the primary consumers of periphyton (primary producer), they also serve as prey to higher trophic-level consumers (predators such as fish or plechopteran genera). In response to predator cues (i.e., top-down cues), grazers change their movements, drift behavior, and diel periodicity of activity (Kohler and McPeek 1989; Miyasaka et al. 2003). The present study revealed that the grazers also changed their behavior on the basis of microalgal cues (i.e., bottom-up cues). Therefore, grazers determine their behaviors to keep a balance between the top-town and bottom-up cues: fitness will be maximized by avoiding predation and obtaining more food resources.

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References

Allan, J. D., and M. M. Castillo. 2007. Stream ecology: Structure and function of running waters, 2nd ed. Springer.

Biggs, B. J. F. 1996. Patterns in benthic algae of streams, p. 31–56. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic.

Bothwell, M. L., K. E. Suzuki, M. K. Bolin, and F. J. Hardy. 1989. Evidence of dark avoidance by phototrophic periphytic diatoms in lotic systems. J. Phycol. **25:** 85–94.

Bowne, D. R., and H. R. White. 2004. Searching strategy of the painted turtle *Chrysemys picta* across spatial scales. Anim. Behav. **68:** 1401–1409.

Brönmark, C., and L. A. Hansson. 2000. Chemical communication in aquatic systems: An introduction. Oikos **88:** 103–109.

Burks, R. L., and D. M. Lodge. 2002. Cued in: Advances and opportunities in freshwater chemical ecology. J. Chem. Ecol. **28:** 1901–1917.

Dahl, J., P. A. Nilsson, and L. B. Pettersson. 1998. Against the flow: Chemical detection of downstream predators in running waters. Proc. R. Soc. London B **265:** 1339–1344.

Demott, W. R. 1986. The role of taste in food selection by freshwater zooplankton. Oecologia **69:** 334–340.

Doi, H., and I. Katano. 2008. Distribution patterns of stream grazers and relationships between grazers and periphyton at multiple spatial scales. J. N. Am. Benthol. Soc. **27:** 295–303.

———, ———, and E. Kikuchi. 2006. The use of algal-mat habitats by aquatic insect grazers: Effects of microalgal cues. Basic Appl. Ecol. **7:** 153–158.

Feminella, J. W., and V. H. Resh. 1990. Hydrologic influences, disturbance, and intraspecific competition in a stream caddisfly population. Ecology **71:** 2083–2094.

Hart, D. D. 1981. Foraging and resource patchiness: Field experiments with a grazing stream insect. Oikos **37:** 46–52.

———, and C. T. Robinson. 1990. Resource limitation in a stream community: Phosphorus enrichment effects on periphyton and grazers. Ecology **71:** 1494–1502.

Hoffman, A. L., J. D. Olden, J. B. Monroe, N. L. Poff, T. Wellnitz, and J. A. Wiens. 2006. Current velocity and habitat patchiness shape stream herbivore movement. Oikos **115:** 358–368.

Katano, I., H. Mitsuhashi, Y. Isobe, and T. Oishi. 2005*a*. Relationships between crawling activity of *Micrasema quadriloba* (Brachycentridae) larvae and amounts of periphyton resource, p. 219–222. *In* K. Tanida and A. Rossiter [eds.], Proceedings of the 11th international symposium on Trichoptera. Tokai Univ. Press.

———, ———, ———, H. Sato, and T. Oishi. 2005*b*. Reach-scale distribution dynamics of a grazing stream insect, *Micrasema quadriloba* Martynov (Brachycentridae, Trichoptera), in relation to current velocity and periphyton abundance. Zool. Sci. **22:** 853–860.

———, ———, ———, ———, and ———. 2007. Group size of feeding stream case-bearing caddisfly grazers and resource abundance. Basic Appl. Ecol. **8:** 269–279.

Kohler, S. L. 1984. Search mechanism of a stream grazer in patchy environments: The role of food abundance. Oecologia **62:** 209–218.

———, and M. A. McPeek. 1989. Predation risk and the foraging behavior of competing stream insects. Ecology **70:** 1811–1825.

Lamberti, G. A., and V. H. Resh. 1983. Stream periphyton and insect herbivores: An experimental study of grazing by a caddisfly population. Ecology **64:** 1124–1135.

Lima, S. L. 1998. Stress and decision-making under the risk of predation: Recent developments from behavioral, reproductive, and ecological perspectives. Adv. Study Behav. **28:** 215–290.

McIntosh, A. R., B. L. Peckarsky, and B. W. Taylor. 1999. Rapid size-specific changes in the drift of *Baetis bicaudatus* (Ephemeroptera) caused by alterations in fish odour concentration. Oecologia **118:** 256–264.

Miyasaka, H., M. Genkai-Kato, N. Kuhara, and S. Nakano. 2003. Predatory fish impact on competition between stream insect grazers: A consideration of behavioral- and density-mediated effects on an apparent coexistence pattern. Oikos **101:** 511–520.

Olden, J. D., A. L. Hoffman, J. B. Monroe, and N. L. Poff. 2004. Movement behaviour and dynamics of an aquatic insect in a stream benthic landscape. Can. J. Zool. **82:** 1135–1146.

Poff, N. L., and J. V. Ward. 1992. Heterogeneous currents and algal resources mediate in situ foraging activity of a mobile stream grazer. Oikos **65:** 465–478.

Steinman, A. D., and G. A. Lamberti. 1996. Biomass and pigments of benthic algae, p. 295–314. *In* F. R. Hauer and G. A. Lamberti [eds.], Methods in stream ecology. Academic.

Turner, A. M., and S. L. Montgomery. 2003. Spatial and temporal scales of predator avoidance: experiments with fish and snails. Ecology **84:** 616–622.

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