



The use of algal-mat habitats by aquatic insect grazers: effects of microalgal cues

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Summary

Microalgal biomass is important for the growth and survival of aquatic insect grazers, and we hypothesized that the abundance of microalgae mediates the habitat-use behavior of aquatic insect grazers in stream ecosystems. To test this question under laboratory conditions, we prepared four types of experimental habitat in an artificial stream: untreated ceramic plates, ceramic plates with thin algal mats, ceramic plates with thick algal mats, and natural stone with algal mats. We compared the upstream movement forward each experimental habitat substrate by caddisfly grazer *Glossosoma* larvae. At day and at night, a significantly greater number of *Glossosoma* larvae were attracted by thin and thick algal mats as well as by natural stones than by the untreated ceramic plate. At night, thick algal mats attracted significantly more larvae than thin algal mats. Thus, *Glossosoma* larvae can recognize and respond to the abundance of microalgae through microalgal cues (chemical and/or algal drift), which induce the movement of *Glossosoma* larvae to habitats with high microalgal biomass. We propose that cues from microalgae are recognized by grazers in stream ecosystems.

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Zusammenfassung

Die Mikroalgen-Biomasse ist für das Wachstum und Überleben von aquatischen phytophagen Insekten wichtig und wir stellten die Hypothese auf, dass die Abundanz von Mikroalgen das Habitatnutzungs-Verhalten von aquatischen phytophagen Insekten in fließenden Ökosystemen vermittelt. Um diese Frage unter Laborbedingungen zu prüfen stellten wir vier Typen von experimentellen Habitaten in einem künstlichen Fließgewässer her: unbehandelte Keramikplatten, Keramikplatten mit dünnen Algenmatten, Keramikplatten mit dicken Algenmatten und natürliche Steine mit Algenmatten. Wir verglichen die Bewegungen stromaufwärts für jedes experimentelle

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Habitatsubstrat bei der phytophagen Köcherfliegenlarve *Glossosoma*. Während des Tages und der Nacht wurde eine signifikant größere Anzahl von *Glossosoma*-Larven von dünnen und dicken Algenmatten sowie von natürlichen Steinen angezogen als von unbehandelten Keramikplatten. Während der Nacht hielten sich auf den dicken Algenmatten signifikant mehr Larven auf als auf den dünnen. Also können *Glossosoma*-Larven die Abundanz von Mikroalgen wahrnehmen und darauf reagieren, aufgrund von Hinweisen auf die Mikroalgen (chemisch oder/und durch Algendrift), die eine Bewegung der *Glossosoma*-Larven hin zu Habitaten mit großer Algenbiomasse induzieren. Wir behaupten, dass die Signale oder Hinweise von Mikroalgen von Phytophagen in Fließgewässern erkannt werden.

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Introduction

Chemical and visual cues play an important role in determining the interactions of aquatic organisms; knowledge of these cues enhances our understanding of the connections between individual behavior and ecosystem functions (Burks & Lodge, 2002; Lima, 1998a, b). Many recent reports have established that chemical and visual cues from predators can induce changes in the life history, morphology, and behavior of prey in freshwater ecosystems (e.g., Dahl, Nilsson, & Pettersson, 1998; Lardner, 2000; McIntosh, Peckarsky, & Taylor, 1999; Takahara, Kohmatsu, Maruyama, & Yamaoka, 2003; Turner, Fetterolf & Bernot, 1999; Turner & Montgomery, 2003). Likewise, chemical cues from prey species mediate predator–prey interactions: Predators are attracted by the low-molecular weight metabolites that are released by wounded or dead and decaying prey in aquatic ecosystems (e.g., Brönmark and Hansson, 2000; Carr, 1988). Moreover, Turner, Bernot, and Boes (2000) suggested that the chemical cue between predator and freshwater snails is affected by the periphyton abundance. Communication cues between herbivores and producers may be important for determining the indirect interactions of aquatic communities like the chemical cue between predator and herbivores. However, very little is known about communication cues that pass between herbivores and microalgae in freshwater ecosystems.

In stream ecosystems, insect grazers (i.e., caddisfly and mayfly larvae) are generally the dominant component of the invertebrate grazer population. Studies have revealed that the distribution of many insect grazers is determined by microalgal biomass (Hart & Robinson, 1990; Hill & Knight, 1987) and that many insect grazers congregate on microalgal mats formed on stones (Hill & Knight, 1988). Thus, we hypothesized that herbivores recognize cues released from the microalgae in stream ecosystems and that the behavior of insect grazers is influenced by the microalgal cues.

Therefore, we investigated the existence of cues from microalgae and their influence on habitat use by the aquatic caddisfly grazer *Glossosoma* larvae. We prepared algal mats of low (thin) and high (thick) algal abundance in a natural stream and measured the differential use of the algal-mat habitats by *Glossosoma* larvae in an artificial stream. We determined whether the benthic microalgal mat influenced *Glossosoma* behavior by chemical and/or visual cues.

Materials and methods

The biology of *Glossosoma*

The larvae of freshwater caddisfly genus *Glossosoma* (Trichoptera: Glossosomatidae) build dome-shaped sand cases and feed on diatoms and fine particulate organic matter on stones; the sessile larvae are found on the surfaces of stones in watercourse riffles (Kuhara, Nakano, & Miyasaka, 2000; Merritt & Cummins, 1996). *Glossosoma* is generally the dominant genus in invertebrate grazer communities on hard substrates in Japanese headwater streams (Kuhara et al., 2000; Miyasaka, Genkai-Kato, Kuhara, & Nakano, 2003). In the Hirose River of our study area, *Glossosoma* larvae were the dominant grazers on stone surfaces.

Habitat-use of *Glossosoma* larvae in a laboratory experiment

We conducted a laboratory experiment to examine whether the behavior of *Glossosoma* larvae was influenced by microalgal cues. First, we prepared four types of substrate with different microalgal abundances: untreated ceramic plates (10 × 10 × 1 cm), thin algal mats (ceramic plates incubated for 5 days in the Hirose River), thick algal mats (ceramic plates incubated for 15 days), and freshly collected natural river stones. Ceramic plates that were not

incubated in the river (i.e., untreated ceramic plates) served as controls. The algal biomass of the plates incubated for 15 days was expected to be higher than that of the plates incubated for 5 days (Lamberti & Resh, 1983). To confirm this, we measured the ash-free dry mass (AFDM) of the microalgal biomass. After each incubation time, the algal mat was harvested from a plate by scraping with a brush, and the algae were filtered using a precombusted (500 °C for 2 h) Whatman GF/F glass filter (Whatman International Ltd., Maidstone, UK). The 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 was calculated as the difference between the dry weight and the ash weight. In addition, samples of the algal mat on the experimental plates and natural stones were used to identify the dominant algal species by a microscope.

Final instar *Glossosoma* larvae were collected during November and December 2003 from mid-stream in the Hirose River at Sendai, Japan (38°15'N, 140°50'E). The average wet weight of a random sample of *Glossosoma* larvae used for the experiment was 7.7 ± 0.5 mg (mean \pm SE, $n = 15$) using the electronic balance. The velocity of the river current was 18.0 ± 1.1 cm s⁻¹ ($n = 10$), as measured above the river-bottom stones using a portable current meter (Tanida, Yamashita, & Rossiter, 1985) on 10 December 2003. The depth of the river at the larvae sampling site was 10.0 ± 1.1 cm ($n = 10$) as measured using a level randomly on 10 December 2003. The density of *Glossosoma* larvae found on river stones at the site was 20.9 ± 1.9 individuals per 100 cm² ($n = 10$). To the counting, we put a 10 × 10 cm quadrat on the approximately center of stone surface area, and collected *Glossosoma* larvae in the quadrat on 10 December 2003. In addition, we collected stones (maximum diameter: 4.5 ± 0.8 cm, mean \pm SE, $n = 10$) from the sampling site to use as the natural stone substrates.

In the laboratory, we constructed four plastic artificial streams (45 × 14.5 × 17 cm; water depth, 12–14 cm). The current in the experimental stream was maintained at 15.7 ± 0.7 cm s⁻¹ (mean \pm SE, $n = 10$) with an aquarium water pump (E-roka PF-200; GEX Co., Osaka, Japan). The current velocity was measured with a portable current meter (Tanida et al., 1985). The current velocity and water depth are approximately close to the above field habitat data. The streams were placed under a 10-h light:14-h dark cycle using a fluorescent lamp, similar to the conditions at the field site in November. The start time of the experiments at

0800. At the beginning of each experiment, two ceramic plates were arranged in contact with each other on the bottom of the experimental stream. One plate, designated the "experimental plate," was one of the four substrates (untreated ceramic plate as a control treatment, thin algal-mat, thick algal-mat, and nine natural river stones as a square pattern) and was placed upstream, toward the aquarium water pump. Another plate, the "initial plate," was an untreated ceramic plate and was placed downstream and adjoined an experimental plate. In the natural stones experiment, the initial plate was piled about 3 cm thick to achieve the same height as the stones. We deposited 15 *Glossosoma* larvae at the center of the initial plate and counted the number of larvae on the experimental plate after 2 h. The density of the larvae in these experiments (15 individuals per 100-cm² plate) was approximately 70% of the density at our sampling site in the Hirose River. The experiments were conducted under both light (day) and dark (night) conditions. All treatments were run in each experimental stream simultaneously.

We evaluated the behavioral responses of *Glossosoma* larvae to these four types of habitat substrate by measuring the proportion of *Glossosoma* larvae that had moved to the experimental plate from the initial plate. For each measurement, we sketched the position of the larvae at the beginning of the experiment and after 2 h. We evaluated the four experimental streams with each substrate and *Glossosoma* larva at the same time with five replicates.

Statistical analysis

We used repeated-measures two-way ANOVA to analyze the effects of the algal abundance (control plate, thin algal-mat, thick algal-mat, and natural stone) and light (day/night) on the use of the experimental habitats by *Glossosoma* larvae. We detected significant treatment effects with Holm's multiple comparison test (Holm, 1979).

Results

Algal mats on test plates

The AFDM of the thick algal mats (2.49 ± 0.75 g m⁻², mean \pm SE, $n = 4$) was significantly higher than that of the thin algal mats (0.78 ± 0.12 g m⁻², $n = 4$) (t -test, $p < 0.05$). The difference reflected the longer incubation time in the river at the field site. The AFDM of the attached organic matter on

the control plates was not detectable on an electronic balance. The AFDM of the natural stones ($10.5 \pm 0.7 \text{ g m}^{-2}$, $n = 4$) was significantly higher than that of the thin and thick algal mats (one-way ANOVA, $p < 0.01$, Holm's multiple comparison, $p < 0.05$).

The dominant algal species of the thin algal mats were *Navicula* spp. from number of individuals. Those of the thick algal mats were *Navicula* spp., *Fagilaria* spp. *Melosira varians*, and those of the natural stones were *Navicula* spp., *Melosira varians*, *Pinnularia* spp. Thus, the algal species composition of the test plates were mainly *Navicula* spp., and the other benthic diatom species.

Habitat use by *Glossosoma* larvae

The analysis using repeated-measures two-way ANOVA showed a significant effect of the substrates ($p < 0.001$) and light (day/night) ($p < 0.05$) on the use of the experimental habitats by *Glossosoma* larvae (Table 1). However, there were no statistically significant interactions between the effects of the substrates and the effects of day/night on the number of larvae that moved to the experimental plates (substrates \times day/night, $p = 0.38$).

Under light (day) condition, significantly more larvae moved from the initial plate to the thin algal-mat, the thick algal-mat, and the stone substrates than to the control plate (Fig. 1A). Although there were no significant differences among the percentage of larvae that moved to the thin algal-mat, the thick algal-mat, and the stone substrates, the percentage that moved to the thick algal-mat substrate ($53.3 \pm 5.8\%$) tended to be higher than the percentage that moved to the thin algal-mat or the stone substrates (Fig. 1A).

Under dark (night) conditions, significantly more larvae moved to the thin algal-mat, the thick algal-mat, and the stone substrates than to the control plate (Fig. 1B). In contrast to the larval movements under light conditions, significantly more larvae moved to the thick algal-mat substrate than to the

Table 1. Repeated-measures two-way ANOVA for the effects of the experimental substrates and day/night conditions on habitat use by *Glossosoma* larvae

Source of variation	df	F	P
Percentage moved to experimental substrates			
Substrate effects	3	11.64	<0.001
Day/night effects	1	4.32	0.045
Substrates \times Day/night	3	1.06	0.38
Error	26		

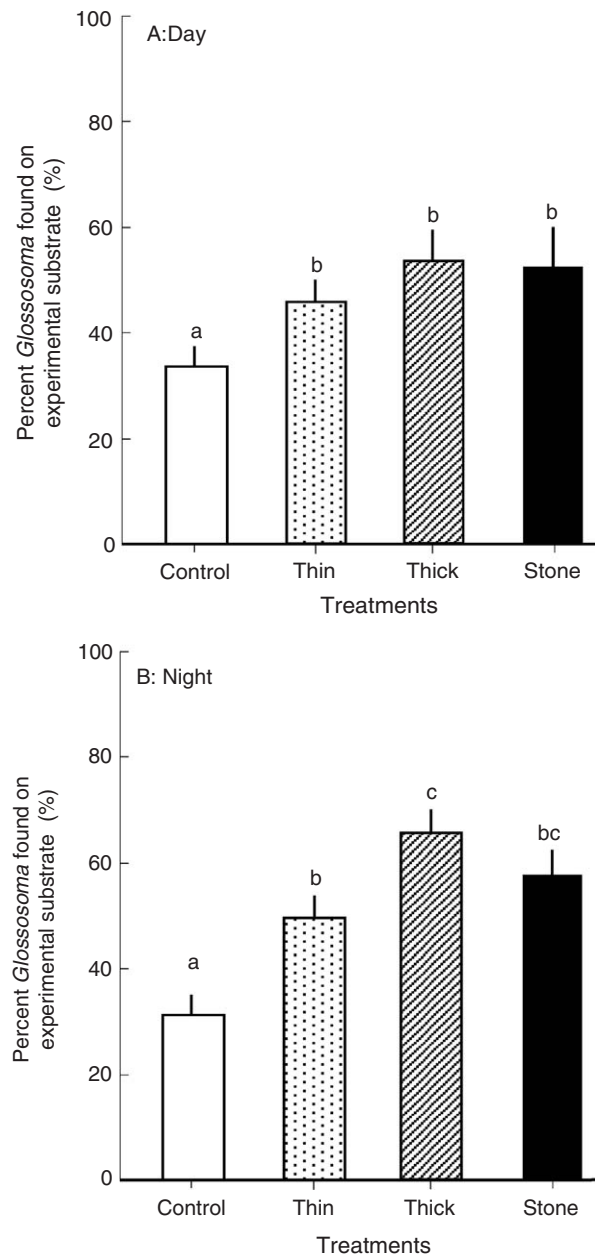


Fig. 1. Behavioral responses of *Glossosoma* larvae to the experimental substrates under light (day; A) and dark (night; B) conditions. The bars indicate the percentages of *Glossosoma* larvae (mean \pm SE, $n = 5$) that moved to the experimental substrates. The letters at the top of each bar indicate significant differences among the experimental conditions (Holm's multiple comparison, $p < 0.05$, $n = 5$).

thin algal-mat substrate (Fig. 1B) under dark conditions. The percentage of *Glossosoma* larvae that moved to the stone substrate was not significantly different from the percentage that moved to the thin or thick algal-mat substrates (Fig. 1B).

Significantly more *Glossosoma* larvae moved to the thick algal-mat plates under the night condition

than under the day condition (t -test, $p < 0.05$, $n = 5$). There were no significant differences in habitat use by *Glossosoma* larvae between the night and day conditions for the other substrates tested (t -test, $p > 0.05$, $n = 5$).

In our experiments, the *Glossosoma* larvae rarely moved off from the initial plates into the downstream direction (~ 2 of 15 individuals), and the larvae that stayed on the initial plates throughout the experiment moved less than 0.5 cm during the 2-h incubation (~ 2 of 15 individuals).

Discussion

More *Glossosoma* larvae moved to the thin algal-mat, the thick algal-mat, or the stone substrates than to the control plates, indicating that the movement of *Glossosoma* larvae was induced by the algal biomass on the experimental plates placed upstream from their starting location. The present results suggest that *Glossosoma* larvae recognized the presence of the microalgae biomass upstream and significantly moved toward it. Moreover, the attraction of the larvae to the thick and thin algal mats was similar to their attraction to freshly collected natural river stones, indicating that the algal mats on the plates can modulate the behavior of *Glossosoma* larvae in a way similar to that of the algae growing on natural stones.

Glossosoma larvae movements in the field are limited to approximately 5 cm h^{-1} (Kuhara, Nakano, & Miyasaka, 2001), and the distance between the starting point on the initial plate and the final location of the larvae on the experimental plate was typically greater than 5 cm (5–15 cm). Therefore, under the conditions of our experiments, about half of the *Glossosoma* larvae would not have had enough time to move to the algal mat on the experimental plates within the 2-h time period.

Our night observations showed that the percentage of *Glossosoma* larvae that moved to the thick algal-mat plates was significantly higher than the percentage that moved to the thin algal-mat plates. The AFDM of the thick and thin algal mats indicated that the abundance of microalgae was much higher in the thick algal mats than in the thin algal mats. Thus, *Glossosoma* larvae might recognize and respond to the abundance of microalgae, moving more frequently to algal mats with higher biomass, and this is for two main reasons for the phenomenon: First, in aquatic ecosystems, chemical cues are thought to be more important in determining the behavior of organisms during darkness because the organisms presumably would

not be able to detect visual cues as well at night (see Burk & Lodge, 2002). Second, some particular diatoms are known to drift in the dark more than in the light (Bothwell, Suzuki, Bolin, & Hardy, 1989), and benthic diatoms were the dominant component of the algal communities on the experimental plates and natural stones. Thus, *Glossosoma* larvae are probably attracted by chemical cues and/or the drift diatoms from the algal mat. Since filter-feeding blackflies have changed their behavior of fan flicking by the concentration of drifting particulate matters (Hart, Merz, Genovese, & Clark, 1991; Schröder, 1987), it is conceivable that the drifting materials including diatoms could affect the behavior of stream insects widely.

A number of field studies have demonstrated that the heterogeneous distributions of various trichopteran grazers are determined by microalgal abundance (e.g., Hart & Robinson, 1990; Hill & Knight, 1987). Our experiments also showed that the movement of *Glossosoma* larvae was influenced by microalgal abundance. Thus, it is probable that the distribution of aquatic insect grazers is determined by microalgal cues in natural streams.

Many studies have suggested that grazers respond to habitats with high food availability (Hart & Robinson, 1990; Hill & Knight, 1987; Lamberti, Feminella, & Resh, 1987). Moreover, low periphyton biomass reduced the growth and survival of trichopteran grazers (Feminella & Resh, 1990). Kuhara et al. (2001) showed that low periphyton availability resulted in an increased distance of *Glossosoma* larval movements, indicating that *Glossosoma* larvae used longer movements to maximize their food intake. In our experiments, *Glossosoma* larvae also moved long distance ($> 10 \text{ cm}^2 \text{ h}^{-1}$) than usual ($\sim 10 \text{ cm}^2 \text{ h}^{-1}$) toward the algal-mat plates in order to maximize their food intake.

Many reports have established that chemical cues from predators mediate the behavior of prey and that communication in predator-prey interactions are often mediated by cues from predators (e.g., Dahl et al., 1998; Takahara et al., 2003; Turner et al., 1999, 2000; Turner & Montgomery, 2003). Our results indicate that cues from microalgae mediated habitat use by *Glossosoma* larvae and that the responses of *Glossosoma* larvae to the microalgal cues were adaptive for their growth and survival.

The present results suggested the importance of microalgal cues for habitat use by lotic insect grazers. Thus, the primary producers such as periphyton mediate the feeding behavior and movement of lotic insects by trait-mediated indirect effects. The present study may be an initial

step toward revealing the importance of communication cues between microalgae and grazers in lotic ecosystems. Further studies are needed to identify the types of microalgal cues that are most important in influencing the behavior of grazers and whether similar microalgal cues affect the behavior of other algal-feeding stream animals.

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References

- Bothwell, M. L., Suzuki, K. E., Bolin, M. K., & Hardy, F. J. (1989). Evidence of dark avoidance by phototrophic periphytic diatoms in lotic systems. *Journal of Phycology*, *25*, 85–94.
- Brönmark, C., & Hansson, L. A. (2000). Chemical communication in aquatic systems: An introduction. *Oikos*, *88*, 103–109.
- Burks, R. L., & Lodge, D. M. (2002). Cued in: Advances and opportunities in freshwater chemical ecology. *Journal of Chemical Ecology*, *28*, 1901–1917.
- Carr, W. E. S. (1988). The molecular nature of chemical stimuli in the aquatic environment. In J. Atema, R. R. Fay, A. N. Popper, & W. N. Tavolga (Eds.), *Sensory biology of aquatic animals* (pp. 3–27). Berlin: Springer.
- Dahl, J., Nilsson, P. A., & Pettersson, L. B. (1998). Against the flow: Chemical detection of downstream predators in running waters. *Proceedings of the Royal Society of London Series B-Biological Sciences*, *265*, 1339–1344.
- Feminella, J. W., & Resh, V. H. (1990). Hydrologic influences, disturbance, and intraspecific competition in a stream caddisfly population. *Ecology*, *71*, 2083–2094.
- Hart, D. D., Merz, R. A., Genovese, S. J., & Clark, B. D. (1991). Feeding postures of suspension-feeding larval black flies: The conflicting demands of drag and food acquisition. *Oecologia*, *85*, 457–467.
- Hart, D. D., & Robinson, C. T. (1990). Resource limitation in a stream community: Phosphorus enrichment effects on periphyton and grazers. *Ecology*, *71*, 1494–1502.
- Hill, W. R., & Knight, A. W. (1987). Experimental analysis of the grazing interaction between a mayfly and stream algae. *Ecology*, *68*, 1955–1965.
- Hill, W. R., & Knight, A. W. (1988). Concurrent grazing effects of two stream insects on periphyton. *Limnology and Oceanography*, *33*, 15–26.
- Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, *6*, 65–70.
- Kuhara, N., Nakano, S., & Miyasaka, H. (2000). Flow rate mediates the competitive influence of a grazing caddisfly on mayflies. *Ecological Research*, *15*, 145–152.
- Kuhara, N., Nakano, S., & Miyasaka, H. (2001). Alterations in the grazing activities of cased caddisfly larvae in response to variations in predation risk and resource level. *Ecological Research*, *16*, 705–714.
- Lamberti, G. A., Feminella, J. W., & Resh, V. H. (1987). Herbivory and intraspecific competition in a stream caddisfly population. *Oecologia*, *73*, 75–81.
- Lamberti, G. A., & Resh, V. H. (1983). Stream periphyton and insect herbivores: An experimental study of grazing by a caddisfly population. *Ecology*, *64*, 1124–1135.
- Lardner, B. (2000). Morphological and life history responses to predators in larvae of seven anurans. *Oikos*, *88*, 169–180.
- Lima, S. L. (1998a). Stress and decision-making under the risk of predation: Recent developments from behavioral, reproductive, and ecological perspectives. *Advances in the Study of Behavior*, *28*, 215–290.
- Lima, S. L. (1998b). Nonlethal effects in the ecology of predator–prey interactions. *Bioscience*, *48*, 25–34.
- McIntosh, A. R., Peckarsky, B. L., & Taylor, B. W. (1999). Rapid size-specific changes in the drift of *Baetis bicaudatus* (Ephemeroptera) caused by alterations in fish odour concentration. *Oecologia*, *118*, 256–264.
- Merritt, R. W., & Cummins, K. W. (1996). *An introduction to the aquatic insects of North America* (3rd ed.). Iowa, USA: Kendall/Hunt Publishing Co.
- Miyasaka, H., Genkai-Kato, M., Kuhara, N., & Nakano, S. (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.
- Schröder, P. (1987). Filter feeding activity of blackfly larvae (Diptera: Simuliidae) in relation to stream velocity and food supply. *Archiv für Hydrobiologie*, *77*, 161–182.
- Takahara, T., Kohmatsu, Y., Maruyama, A., & Yamaoka, R. (2003). Effects of fish chemical cues on tadpole survival. *Ecological Research*, *18*, 793–796.
- Tanida, K., Yamashita, K., & Rossiter, A. (1985). A portable current meter for field use. *Japanese Journal of Limnology*, *46*, 219–221.
- Turner, A. M., Bernot, R. J., & Boes, C. M. (2000). Chemical cues modify species interactions: The ecological consequences of predator avoidance by freshwater snails. *Oikos*, *88*, 148–158.
- Turner, A. M., Fetterolf, S. A., & Bernot, R. J. (1999). Predator identity and consumer behavior: Differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia*, *118*, 242–247.
- Turner, A. M., & Montgomery, S. L. (2003). Spatial and temporal scales of predator avoidance: Experiments with fish and snails. *Ecology*, *84*, 616–622.