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Stream grazers determine their crawling direction on the basis of chemical and visual/tactile microalgal cues

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Abstract

This study aimed to determine the association of herbivore behavior with cues from producers. We used stream grazer *Glossosoma* larvae and determined their crawling direction in relation to the chemical and visual cues from microalgae. The experimental treatments included control (no cue), particulate (chemical and visual/tactile cues), and dissolved (chemical cue) cues from microalgae. The experimental water samples were randomly placed into either arms of a Y-shaped channel, and the crawling direction of the grazers was determined. Although the grazers crawled toward the arm containing either particulate or dissolved cues, they preferred the arm with particulate cues. This suggested that grazers responded well when both visual/tactile (i.e., drifting algal cells) and chemical cues (algal smell) were present, and that visual/tactile cues were more important for foraging. In natural habitats, grazers detect cues from both producers and predators and use them to maximize fitness by avoiding predation and obtaining food.
Chemical and visual cues play important roles in species interactions (Lima 1998a,b; Burks & Lodge 2002). Numerous studies have established that these cues are also important in predator–prey interactions (Carr 1998; Turner, Fetterolf & Bernot 1999; Brönmark & Hansson 2000; Turner, Bernot & Boes 2000; Turner & Montgomery 2003; Takahara et al., 2012; Takahara et al., 2013). Predators can be attracted to food patches by recognizing cues from animal prey (Carr 1998; Burks & Lodge 2002).

The interaction of cues also occurs between predators (herbivores) and primary producers. Many studies have shown that morphological changes occur in primary producers on the basis of cues from predators (Larsson & Dodson 1993; Brönmark & Hansson 2000; Lürling & Von Elert 2001). However, few studies have investigated the herbivore responses to producer cues, and these studies have been limited to consumption (Poulet & Marsot 1978; DeMott 1986), habitat use (Doi, Katano & Kikuchi 2006), and foraging behavior (Katano, Doi & Oishi 2009).

In stream ecosystems, grazers need to effectively respond to various heterogeneous periphyton resources because periphyton is patchily distributed (Biggs 1996). Various grazers perform area-restricted searches (Krebs 1978) to obtain abundant food resources (Hart 1981; Kohler 1984). Caddisfly and mayfly grazers utilize alternative behaviors
depending on periphyton abundance; they move rapidly until they arrive at a patch having abundant periphyton and then move slowly within the patch (the former is an extensive form of search and, the latter, intensive [Kohler 1984; Poff & Ward 1992; Katano et al. 2005; Hoffman et al., 2006]).

Doi et al., (2006) and Katano et al., (2009) hypothesized that cues from periphyton (i.e., microalgal cues) mediate the interaction between grazers and periphyton. The presence of microalgal cues was confirmed by the changes in the habitat use and searching behavior of Glossosoma larvae that detected and responded to the microalgal cues. However, they did not determine whether the microalgal cues were chemical, visual, or tactile. Diatoms are generally known to drift from periphyton mats (Bothwell et al., 1989; Stevenson & Peterson 1991), and such drifting diatoms might function as microalgal cues for herbivores. Diatoms are known to drift more at night than during the day (Bothwell et al., 1989) and Doi et al., (2006) reported that the habitat use of Glossosoma larvae in patches with abundant periphyton was greater at night than during the day. On the basis of this finding, we speculated that the larvae might detect microalgae (i.e., flowing microalgal cells) via visual and/or tactile cues, although differentiating between visual and tactile cues might be difficult (hence, hereafter, we use visual/tactile cues for microalgal cues).

However, this hypothesis has never been tested, although knowledge about cue mechanisms (chemical or visual/tactile) is important for completely understanding the
interaction of cues between producers and stream herbivores.

In this study, we performed a laboratory experiment to determine whether microalgal cues (chemical or visual/tactile or both) play an important role in the interaction between periphyton and grazers by using the larvae of caddisfly grazer, *Glossosoma* sp. (Trichoptera: Glossosomatidae). The genus *Glossosoma* includes grazer species that feed on periphyton. The larvae of these species build dome-shaped sand cases and crawl on the surfaces of stones in riffles (Feminella & Hawkins 1995; Merritt & Cummins 1996).

*Glossosoma* larvae are often the dominant grazers in Japanese streams with hard substrates (Doi & Katano 2008).

Materials and Methods

Collection of *Glossosoma* larvae and experimental water

In June 2006, we collected 60 cobbles to acquire periphyton, *Glossosoma* larvae (2.3 ± 0.9 mg dry mass), and river water from riffles in the Agi-gawa River, a tributary of the Kiso-gawa River system, Gifu Prefecture, Japan (35°26′49″N, 137° 25′12″E). Periphyton were brushed off the cobbles and placed in a container with 1 L of surface river water. The container and living larvae were transported in an aerated cooler to the laboratory. No
Specific permits were required for the field studies described, because the location was not privately owned or protected, and the field studies did not involve capturing endangered or protected species.

In the laboratory, we prepared three types of experimental water treatments: filtered river water as a control, dissolved microalgal cue water, and particulate microalgal cue water. For the control, 60 L of Agi-gawa river water was filtered through a GA-100 glass filter (Toyo-roshi Co., Tokyo, Japan; pore size, 1 µm). Particulate microalgal cue water was obtained by adding half of the well-mixed periphyton suspension water into 60 L of filtered river water. For the dissolved microalgal cue water, the other half of the well-mixed periphyton suspension was passed through a glass filter (GA-100, Toyo-roshi Co., Tokyo, Japan; pore size, 1 µm) and added to 60 L of filtered river water.

In the experiment, we assumed the materials in the experiment waters as follows. The control water only contained the cues from only surface water. The dissolved water contained the extra celllar cues from periphyton, such as the chemicals from the algal cells, but did not contain the algal cells due to the small pore size of the filter (1µm). The particulate water contained all materials from periphyton including the cells and chemicals.

The abundance of microalgae cells in each experimental water was estimated by
measuring chlorophyll a (Chl a) concentration. If the experiment water only contained the chemicals not the algal cells, the Chl a concentration would be negligibly low. Well-mixed experimental water (100 mL per treatment) was filtered through a glass filter (GA-100, Toyo-roshi Co., Tokyo, Japan; pore size, 1 µm). The filter was then cut into small pieces and placed into vials, each containing 20 mL of 99.5% ethanol. The vials were preserved in the dark at 4°C for 24 h; subsequently, the extracted pigments were measured using a MPS-2000 spectrophotometer (Shimadzu Co., Japan). The Chl a contents were determined according to the method of UNESCO (UNESCO 1966), and then the abundance of microalgae in the experimental water was assessed (mg Chl a·m⁻³).

Experiment channel

A Y-shaped, one-way experimental channel (branched zone: 20 cm long × 2.5 cm wide × 0.5–0.7 cm water depth; experimental zone: 25 cm long × 2.7 cm wide × 0.7–1 cm water depth) was used for the experiment (Fig. 1). Two types of experimental water were randomly placed in either the left or right arm of the water tank. The current velocity at the start line was maintained at 8.3 ± 1.8 cm·s⁻¹ (mean ± 1 SD, n = 48) by using two aquarium water pumps (Rio-1100; Kamihata Fish Industry Co., Himeji, Japan). The discharge from the left and right pumps to the branched channels ranged from 3.7 to 8.0 mL·s⁻¹ and from
3.9 to 8.4 mL·s⁻¹ respectively. Analysis of covariance (ANCOVA) showed that the discharge from the left and right pumps was not significantly different ($F = 0.42, p = 0.40, n = 48$); further, there was no significant interaction between the discharge and the water in the tanks ($F = 0.42, p = 0.52$). During the experiment, discharge from both the pumps to the branched (i.e., left and right) channels was repeatedly measured. An increase in the water temperature in the experimental tanks was avoided by placing gel ice packs in the tanks, if necessary; the water temperature was maintained between 17.2 and 20.9°C, which was within the diurnal fluctuation range of the Agi-gawa River during the study period.

At the beginning of each trial, a randomly selected *Glossosoma* larva was placed at the start line (Fig. 1). The trial was finished when the larva reached either goal line, and then the larva was allowed to select between the two types of experimental waters, which flowed through the attained goal line (Fig. 1). We randomly performed six experimental combinations with three types of experimental water (control, particulate, and dissolved microalgal cues) and left/right water tanks. An experimental set, which consisted of eight individual larval replicates ($n = 8$), was repeated 5 times; in all, 240 trials were conducted.

This study was performed in strict accordance with the recommendations of the Guidelines for the Proper Conduct of Animal Experiments by the Science Council of Japan.
(Jan 2006). According to the guidelines, a special permission was not required for conducting experiments on invertebrates. All efforts were made to minimize suffering.

Statistical analysis

Three types of combinations (i.e., control vs. dissolved microalgal cue, control vs. particulate microalgal cue, and dissolved vs. particulate microalgal cue) were used to test the selections by larvae of either experimental water by using the binomial exact test. The proportion of numbers of *Glossosoma* larvae among the treatments was tested using Newcombe’s test of equal proportions to test the null hypothesis that the proportions in several groups are the same. For considering multiple comparisons for Type I error, the significances of Newcombe’s test among the treatments were fixed using a Bonferroni criteria. The difference in periphyton cell abundance of the experimental water was tested using one-way analysis of variance (ANOVA), and post-hoc multiple comparisons were performed using Tukey’s test. For all statistical analyses, $\alpha = 0.05$ was used as the significance criterion. All statistical analyses were performed using R version 2.15.3 (R Development Core Team 2013).
Results

Chl \( a \) concentrations were significantly different among the control, dissolved, and particulate microalgal cue waters (one-way ANOVA, \( F = 20.75, p < 0.001 \)). Microalgal abundance was not significantly different between the control and dissolved cue waters (0.8 ± 1.5 and 5.0 ± 2.5 mg Chl \( a \) \( \cdot \) m\(^{-3} \), respectively; Tukey’s test, \( p > 0.05 \)), and was significantly higher for the water with particulate cues (21.4 ± 8.8 mg Chl \( a \) \( \cdot \) m\(^{-3} \), \( p < 0.05 \)) than of those of the remaining two waters. As we expected, the experiment water with only the chemicals (control and dissolved cue waters) had lower Chl \( a \) concentration.

Significantly higher numbers of *Glossosoma* larvae selected particulate microalgal cue water over control river water (binomial exact test, \( p < 0.0001 \); Fig. 2). In addition, the numbers of larvae that selected dissolved cue water were almost significantly higher than those that selected control water (\( p = 0.03 \)). However, there was no significant difference in the numbers of larvae that selected either particulate or dissolved cue water (\( p = 0.11 \)). The proportions of *Glossosoma* larvae were significantly different among the treatments (Newcombe’s test of equal proportions with Bonferroni criteria, \( p < 0.05 \)), indicating that the preference for water with particulate cues over control was significantly higher than that for water with dissolved cues.
We found that *Glossosoma* larvae preferred both particulate and dissolved cues (i.e., visual/tactile and chemical cues and chemical cue only, respectively) over the control water. As we hypothesized, the both cues are important to determine the foraging direction of the grazer, *Glossosoma* larvae. Preference for particulate cues over the control was significantly higher than that for dissolved cues over the control, indicating that *Glossosoma* larvae responded well when both visual/tactile (i.e., detecting drifting microalgae) and chemical cues were present. *Glossosoma* larvae could probably detect materials drifting from upstream by using their eyes and/or tactile organs. In addition, the present study revealed that the larvae could determine the crawling direction for a suitable food source by using microalgal cues. Accurately determining the crawling direction might result in efficient feeding for facilitating growth (Lamberti & Resh 1983; Feminella & Resh 1990; Hart & Robinson 1990).

The following could be the merits of detecting both chemical and visual cues from microalgae: (1) Periphyton, especially some diatoms, are known to drift from periphyton mats (Bothwell et al., 1989; Stevenson & Peterson 1991), and periphytic cells are removed...
and then suspended by current turbulence in streams. Thus, numerous visual/tactile cues would be present in the stream water, that is, visual cues from periphyton mats (i.e., suspended algal cells in the water), for deciding the search direction. (2) Chemical cues might not necessarily be transmitted from upstream because, in stream water, chemical cues would be diffused not only downstream but also in other directions. Therefore, grazers would benefit if they can detect visual/tactile cues as well to adjust their search direction, which was initially determined using chemical cues.

In this study, we conducted a simple experiment to evaluate the effects of chemical and visual/tactile cues on Glossosoma grazers. Although this is an initial step toward understanding the cue interaction between producers and grazers, there were a few limitations to this study. First, we did not evaluate the differences in the concentrations of chemical cues for determining the crawling direction. Katano et al., (2009) suggested that an increase in algal cues induces responses in grazer behavior. Thus, increasing microalgal (chemical and visual/tactile) cues would influence the determination of the crawling direction; for instance, cues arising from smaller algal biomass might not direct grazers toward suitable food patches. Second, in this study, we assumed that grazers would detect both chemical and visual/tactile cues from the experimental water containing dissolved and particulate cues. However, the compositions of microalgal cues (i.e., microalgal cells,
materials, species, and dissolved cues) in both the experimental water treatments were not known. Further study is needed to determine the cue materials or algal species that are important for determining the behavior of grazers.

In this study, we found that both chemical and visual/tactile cues from microalgae were useful for the grazers. However, previous studies have shown that prey that changed their activity to avoid predation by detecting only the chemical cues from the predators (Kuhara, Nakano & Miyasaka 2001; Miyasaka et al., 2003). Both sensitivities for chemical and visual cues would be caused in the difference of their lethality. For example, prey need to rapidly and precisely detect predator chemical cues to effectively avoid the lethal effects of predators (McIntosh, Peckarsky & Taylor 1999; Tuner & Montgomery 2003). On the other hand, macroinvertebrate grazers (i.e., predators of periphyton) would not be at a risk of starving to death if they required time to reach a patch with abundant periphyton (i.e., prey of grazers). Thus, grazers could efficiently select the direction of crawling toward a suitable periphyton patch by using both chemical and visual/tactile cues. Since microalgae such as diatoms usually drift in field streams (Bothwell et al., 1989) like particulate microalgal cues, the cues from dissolved matter and particulate matter would provide the grazers with an opportunity to effectively search for food patches.
Glossosoma grazers are the primary consumers of periphyton (primary producer) and serve as prey to higher-trophic-level consumers (predators such as fish and/or plecopteran genera). In response to predator cues (i.e., top-down cues), grazers change their movements, drift behavior, and diel periodicity of activity (Kohler & McPeek 1989; Kuhara, Nakano & Miyasaka 2001; Miyasaka et al., 2003). The present study revealed that grazers also changed their behavior on the basis of microalgal cues (i.e., bottom-up cues). Therefore, grazers change their behaviors to maintain a balance between the top-down and bottom-up cues, thereby maximizing fitness by avoiding predation and more efficiently obtaining food resources.

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Figure 1. Top views of the experimental channel. Arrows and P show the water flows and water pump, respectively.
Figure 2. The results for selectivity of the two types of experimental water by the test larvae (n = 80 for each combination). ****, *, and NS show p < 0.0001, <0.05, and >0.05 by binomial exact test, respectively. All proportions were significantly different among the treatments (Newcombe’s test with Bonferroni criteria, p < 0.05).