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

4

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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.

36

## Introduction

37

38 Chemical and visual cues play important roles in species interactions (Lima 1998a,b; Burks  
39 & Lodge 2002). Numerous studies have established that these cues are also important in  
40 predator–prey interactions (Carr 1998; Turner, Fetterolf & Bernot 1999; Brönmark &  
41 Hansson 2000; Turner, Bernot & Boes 2000; Turner & Montgomery 2003; Takahara et al.,  
42 2012; Takahara et al., 2013). Predators can be attracted to food patches by recognizing cues  
43 from animal prey (Carr 1998; Burks & Lodge 2002).

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

51 In stream ecosystems, grazers need to effectively respond to various heterogeneous  
52 periphyton resources because periphyton is patchily distributed (Biggs 1996). Various  
53 grazers perform area-restricted searches (Krebs 1978) to obtain abundant food resources  
54 (Hart 1981; Kohler 1984). Caddisfly and mayfly grazers utilize alternative behaviors

55 depending on periphyton abundance; they move rapidly until they arrive at a patch having  
56 abundant periphyton and then move slowly within the patch (the former is an extensive  
57 form of search and, the latter, intensive [Kohler 1984; Poff & Ward 1992; Katano et al.  
58 2005; Hoffman et al., 2006]).

59 Doi et al., (2006) and Katano et al., (2009) hypothesized that cues from periphyton  
60 (i.e., microalgal cues) mediate the interaction between grazers and periphyton. The  
61 presence of microalgal cues was confirmed by the changes in the habitat use and searching  
62 behavior of *Glossosoma* larvae that detected and responded to the microalgal cues.  
63 However, they did not determine whether the microalgal cues were chemical, visual, or  
64 tactile. Diatoms are generally known to drift from periphyton mats (Bothwell et al., 1989;  
65 Stevenson & Peterson 1991), and such drifting diatoms might function as microalgal cues  
66 for herbivores. Diatoms are known to drift more at night than during the day (Bothwell et  
67 al., 1989) and Doi et al., (2006) reported that the habitat use of *Glossosoma* larvae in  
68 patches with abundant periphyton was greater at night than during the day. On the basis of  
69 this finding, we speculated that the larvae might detect microalgae (i.e., flowing microalgal  
70 cells) via visual and/or tactile cues, although differentiating between visual and tactile cues  
71 might be difficult (hence, hereafter, we use visual/tactile cues for microalgal cues).  
72 However, this hypothesis has never been tested, although knowledge about cue  
73 mechanisms (chemical or visual/tactile) is important for completely understanding the

74 interaction of cues between producers and stream herbivores.

75 In this study, we performed a laboratory experiment to determine whether  
76 microalgal cues (chemical or visual/tactile or both) play an important role in the interaction  
77 between periphyton and grazers by using the larvae of caddisfly grazer, *Glossosoma* sp.  
78 (Trichoptera: Glossosomatidae). The genus *Glossosoma* includes grazer species that feed  
79 on periphyton. The larvae of these species build dome-shaped sand cases and crawl on the  
80 surfaces of stones in riffles (Feminella & Hawkins 1995; Merritt & Cummins 1996).  
81 *Glossosoma* larvae are often the dominant grazers in Japanese streams with hard substrates  
82 (Doi & Katano 2008).

83

## 84 Materials and Methods

85

### 86 Collection of *Glossosoma* larvae and experimental water

87

88 In June 2006, we collected 60 cobbles to acquire periphyton, *Glossosoma* larvae ( $2.3 \pm 0.9$   
89 mg dry mass), and river water from riffles in the Agi-gawa River, a tributary of the  
90 Kiso-gawa River system, Gifu Prefecture, Japan ( $35^{\circ}26'49''\text{N}$ ,  $137^{\circ}25'12''\text{E}$ ). Periphyton  
91 were brushed off the cobbles and placed in a container with 1 L of surface river water. The  
92 container and living larvae were transported in an aerated cooler to the laboratory. No

93 specific permits were required for the field studies described, because the location was not  
94 privately owned or protected, and the field studies did not involve capturing endangered or  
95 protected species.

96

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

105 In the experiment, we assumed the materials in the experiment waters as follows. The  
106 control water only contained the cues from only surface water. The dissolved water  
107 contained the extra cellular cues from periphyton, such as the chemicals from the algal cells,  
108 but did not contain the algal cells due to the small pore size of the filter (1  $\mu\text{m}$ ). The  
109 particulate water contained all materials from periphyton including the cells and chemicals.

110

111 The abundance of microalgae cells in each experimental water was estimated by

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

#### 122 Experiment channel

123  
124 A Y-shaped, one-way experimental channel (branched zone: 20 cm long  $\times$  2.5 cm wide  $\times$   
125 0.5–0.7 cm water depth; experimental zone: 25 cm long  $\times$  2.7 cm wide  $\times$  0.7–1 cm water  
126 depth) was used for the experiment (Fig. 1). Two types of experimental water were  
127 randomly placed in either the left or right arm of the water tank. The current velocity at the  
128 start line was maintained at  $8.3 \pm 1.8 \text{ cm} \cdot \text{s}^{-1}$  (mean  $\pm$  1 SD,  $n = 48$ ) by using two aquarium  
129 water pumps (Rio-1100; Kamihata Fish Industry Co., Himeji, Japan). The discharge from  
130 the left and right pumps to the branched channels ranged from 3.7 to 8.0  $\text{mL} \cdot \text{s}^{-1}$  and from



131 3.9 to 8.4 mL·s<sup>-1</sup> respectively. Analysis of covariance (ANCOVA) showed that the  
132 discharge from the left and right pumps was not significantly different ( $F = 0.42$ ,  $p = 0.40$ ,  
133  $n = 48$ ); further, there was no significant interaction between the discharge and the water in  
134 the tanks ( $F = 0.42$ ,  $p = 0.52$ ). During the experiment, discharge from both the pumps to the  
135 branched (i.e., left and right) channels was repeatedly measured. An increase in the water  
136 temperature in the experimental tanks was avoided by placing gel ice packs in the tanks, if  
137 necessary; the water temperature was maintained between 17.2 and 20.9°C, which was  
138 within the diurnal fluctuation range of the Agi-gawa River during the study period.

139

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

147 This study was performed in strict accordance with the recommendations of the  
148 Guidelines for the Proper Conduct of Animal Experiments by the Science Council of Japan

149 (Jan 2006). According to the guidelines, a special permission was not required for  
150 conducting experiments on invertebrates. All efforts were made to minimize suffering.

151

152 Statistical analysis

153

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

166

## Results

167

168

169 Chl *a* concentrations were significantly different among the control, dissolved, and  
170 particulate microalgal cue waters (one-way ANOVA,  $F = 20.75$ ,  $p < 0.001$ ). Microalgal  
171 abundance was not significantly different between the control and dissolved cue waters ( $0.8$   
172  $\pm 1.5$  and  $5.0 \pm 2.5$  mg Chl *a*·m<sup>-3</sup>, respectively; Tukey's test,  $p > 0.05$ ), and was  
173 significantly higher for the water with particulate cues ( $21.4 \pm 8.8$  mg Chl *a*·m<sup>-3</sup>,  $p < 0.05$ )  
174 than of those of the remaining two waters. As we expected, the experiment water with only  
175 the chemicals (control and dissolved cue waters) had lower Chl *a* concentration.

176

177 Significantly higher numbers of *Glossosoma* larvae selected particulate microalgal  
178 cue water over control river water (binomial exact test,  $p < 0.0001$ ; Fig. 2). In addition, the  
179 numbers of larvae that selected dissolved cue water were almost significantly higher than  
180 those that selected control water ( $p = 0.03$ ). However, there was no significant difference in  
181 the numbers of larvae that selected either particulate or dissolved cue water ( $p = 0.11$ ). The  
182 proportions of *Glossosoma* larvae were significantly different among the treatments  
183 (Newcombe's test of equal proportions with Bonferroni criteria,  $p < 0.05$ ), indicating that  
184 the preference for water with particulate cues over control was significantly higher than that  
185 for water with dissolved cues.

186

187

## Discussion

188

189 We found that *Glossosoma* larvae preferred both particulate and dissolved cues (i.e.,  
190 visual/tactile and chemical cues and chemical cue only, respectively) over the control water.

191 As we hypothesized, the both cues are important to determine the foraging direction of the

192 grazer, *Glossosoma* larvae. Preference for particulate cues over the control was

193 significantly higher than that for dissolved cues over the control, indicating that

194 *Glossosoma* larvae responded well when both visual/tactile (i.e., detecting drifting

195 microalgae) and chemical cues were present. *Glossosoma* larvae could probably detect

196 materials drifting from upstream by using their eyes and/or tactile organs. In addition, the

197 present study revealed that the larvae could determine the crawling direction for a suitable

198 food source by using microalgal cues. Accurately determining the crawling direction might

199 result in efficient feeding for facilitating growth (Lamberti & Resh 1983; Feminella & Resh

200 1990; Hart & Robinson 1990).

201

202 The following could be the merits of detecting both chemical and visual cues from

203 microalgae: (1) Periphyton, especially some diatoms, are known to drift from periphyton

204 mats (Bothwell et al., 1989; Stevenson & Peterson 1991), and periphytic cells are removed

205 and then suspended by current turbulence in streams. Thus, numerous visual/tactile cues  
206 would be present in the stream water, that is, visual cues from periphyton mats (i.e.,  
207 suspended algal cells in the water), for deciding the search direction. (2) Chemical cues  
208 might not necessarily be transmitted from upstream because, in stream water, chemical cues  
209 would be diffused not only downstream but also in other directions. Therefore, grazers  
210 would benefit if they can detect visual/tactile cues as well to adjust their search direction,  
211 which was initially determined using chemical cues.

212  
213 In this study, we conducted a simple experiment to evaluate the effects of chemical  
214 and visual/tactile cues on *Glossosoma* grazers. Although this is an initial step toward  
215 understanding the cue interaction between producers and grazers, there were a few  
216 limitations to this study. First, we did not evaluate the differences in the concentrations of  
217 chemical cues for determining the crawling direction. Katano et al., (2009) suggested that  
218 an increase in algal cues induces responses in grazer behavior. Thus, increasing microalgal  
219 (chemical and visual/tactile) cues would influence the determination of the crawling  
220 direction; for instance, cues arising from smaller algal biomass might not direct grazers  
221 toward suitable food patches. Second, in this study, we assumed that grazers would detect  
222 both chemical and visual/tactile cues from the experimental water containing dissolved and  
223 particulate cues. However, the compositions of microalgal cues (i.e., microalgal cells,

224 materials, species, and dissolved cues) in both the experimental water treatments were not  
225 known. Further study is needed to determine the cue materials or algal species that are  
226 important for determining the behavior of grazers.

227

228 In this study, we found that both chemical and visual/tactile cues from microalgae  
229 were useful for the grazers. However, previous studies have shown that prey that changed  
230 their activity to avoid predation by detecting only the chemical cues from the predators  
231 (Kuhara, Nakano & Miyasaka 2001; Miyasaka et al., 2003). Both sensitivities for chemical  
232 and visual cues would be caused in the difference of their lethality. For example, prey need  
233 to rapidly and precisely detect predator chemical cues to effectively avoid the lethal effects  
234 of predators (McIntosh, Peckarsky & Taylor 1999; Tuner & Montgomery 2003). On the  
235 other hand, macroinvertebrate grazers (i.e., predators of periphyton) would not be at a risk  
236 of starving to death if they required time to reach a patch with abundant periphyton (i.e.,  
237 prey of grazers). Thus, grazers could efficiently select the direction of crawling toward a  
238 suitable periphyton patch by using both chemical and visual/tactile cues. Since microalgae  
239 such as diatoms usually drift in field streams (Bothwell et al., 1989) like particulate  
240 microalgal cues, the cues from dissolved matter and particulate matter would provide the  
241 grazers with an opportunity to effectively search for food patches.

242

243 *Glossosoma* grazers are the primary consumers of periphyton (primary producer)  
244 and serve as prey to higher-trophic-level consumers (predators such as fish and/or  
245 plecopteran genera). In response to predator cues (i.e., top-down cues), grazers change their  
246 movements, drift behavior, and diel periodicity of activity (Kohler & McPeck 1989; Kuhara,  
247 Nakano & Miyasaka 2001; Miyasaka et al., 2003). The present study revealed that grazers  
248 also changed their behavior on the basis of microalgal cues (i.e., bottom-up cues).  
249 Therefore, grazers change their behaviors to maintain a balance between the top-down and  
250 bottom-up cues, thereby maximizing fitness by avoiding predation and more efficiently  
251 obtaining food resources.

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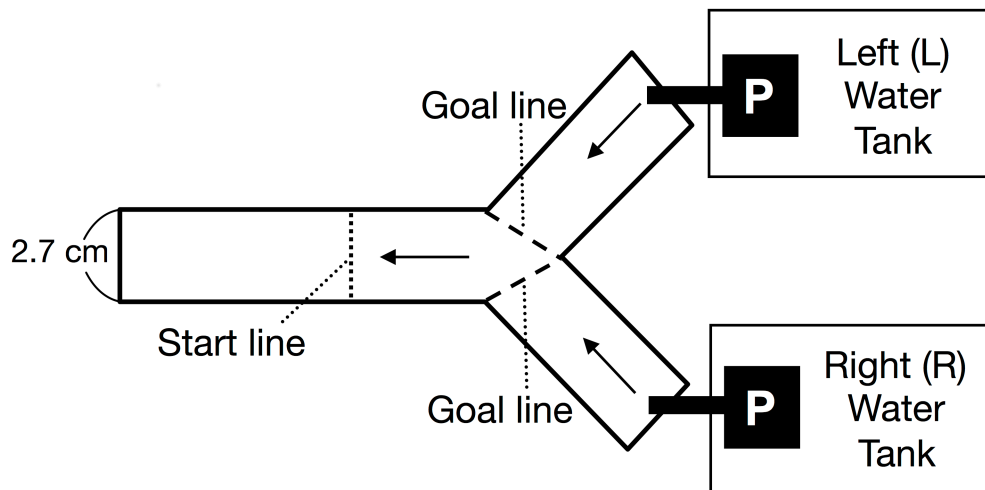
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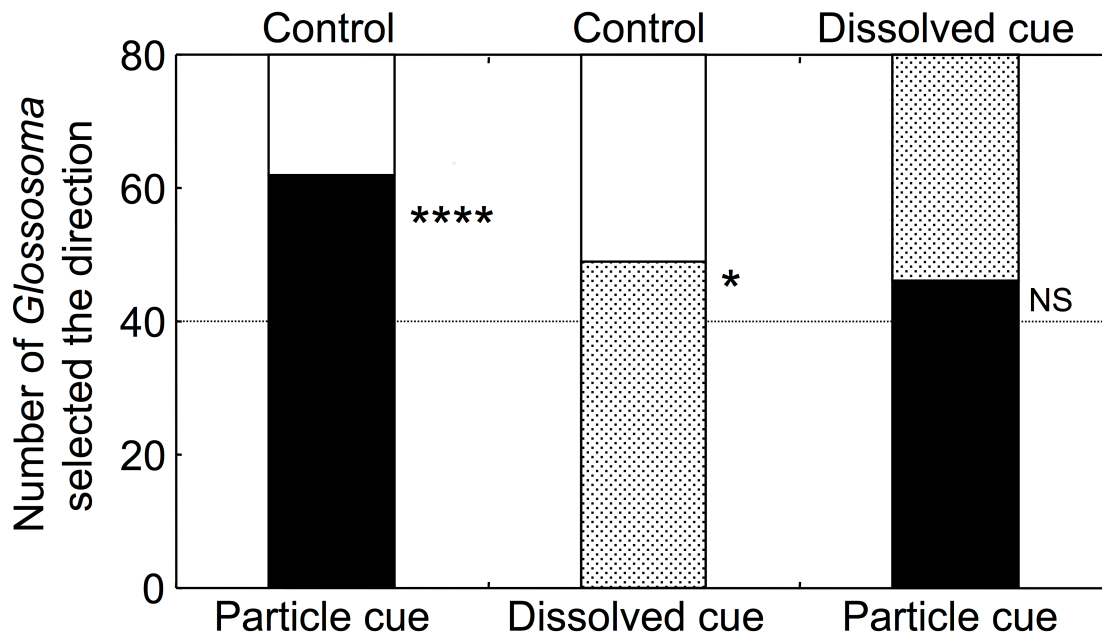
356 Figure 1. Top views of the experimental channel. Arrows and P show the water flows and

357 water pump, respectively.

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360



361

362 Figure 2. The results for selectivity of the two types of experimental water by the test larvae

363 ( $n = 80$  for each combination). \*\*\*\*, \*, and NS show  $p < 0.0001$ ,  $<0.05$ , and  $>0.05$  by

364 binomial exact test, respectively. All proportions were significantly different among the

365 treatments (Newcombe's test with Bonferroni criteria,  $p < 0.05$ ).

366

367