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

3   microalgal cues

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1

20   Abstract

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22   This study aimed to determine the association of herbivore behavior with cues from

23   producers. We used stream grazer *Glossosoma* larvae and determined their crawling

24   direction in relation to the chemical and visual cues from microalgae. The experimental

25   treatments included control (no cue), particulate (chemical and visual/tactile cues), and

26   dissolved (chemical cue) cues from microalgae. The experimental water samples were

27   randomly placed into either arms of a Y-shaped channel, and the crawling direction of the

28   grazers was determined. Although the grazers crawled toward the arm containing either

29   particulate or dissolved cues, they preferred the arm with particulate cues. This suggested

30   that grazers responded well when both visual/tactile (i.e., drifting algal cells) and chemical

31   cues (algal smell) were present, and that visual/tactile cues were more important for

32   foraging. In natural habitats, grazers detect cues from both producers and predators and use

33   them to maximize fitness by avoiding predation and obtaining food.

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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

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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

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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).

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84   Materials and Methods

85

86   Collection of *Glossosoma* larvae and experimental water

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88   In June 2006, we collected 60 cobbles to acquire periphyton, *Glossosoma* larvae (2.3 ± 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 (35º26′49″N, 137º 25′12″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

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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.

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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 μm). 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 μm) 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 cellar 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 (1μm). The

109   particulate water contained all materials from periphyton including the cells and chemicals.

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

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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 μm). 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 4ºC 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 (mg Chl $a \cdot m^{-3}$).

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122   Experiment channel

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124   A Y-shaped, one-way experimental channel (branched zone: 20 cm long × 2.5 cm wide ×

125   0.5–0.7 cm water depth; experimental zone: 25 cm long × 2.7 cm wide × 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 ± 1.8 $cm \cdot s^{-1}$ (mean ± 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 $mL \cdot s^{-1}$ and from

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131   3.9 to 8.4 mL·s^{-1} 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 20.9ºC, 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

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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.

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152   Statistical analysis

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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).

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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　　± 1.5 and 5.0 ± 2.5 mg Chl $a \cdot m^{-3}$, respectively; Tukey's test, $p > 0.05$), and was

173　　significantly higher for the water with particulate cues (21.4 ± 8.8 mg Chl $a \cdot m^{-3}$, $p < 0.05$)

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.

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187   Discussion

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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

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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,

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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.

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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.

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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 & McPeek 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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253   Acknowledgments

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258   Reference

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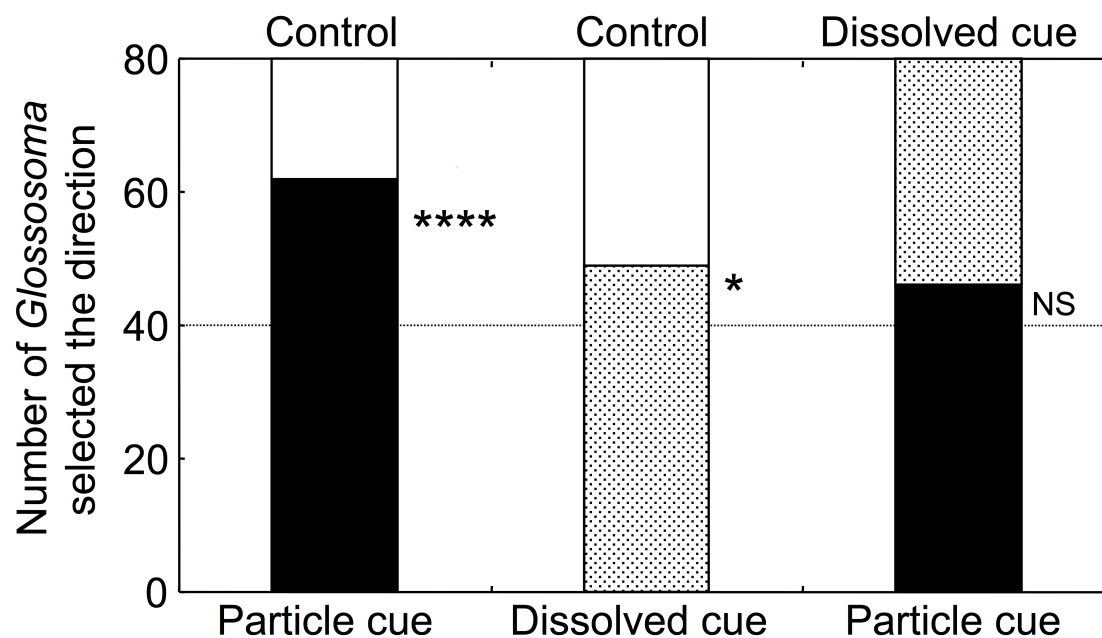351   Monographs in Oceanography Methodology 1, 69

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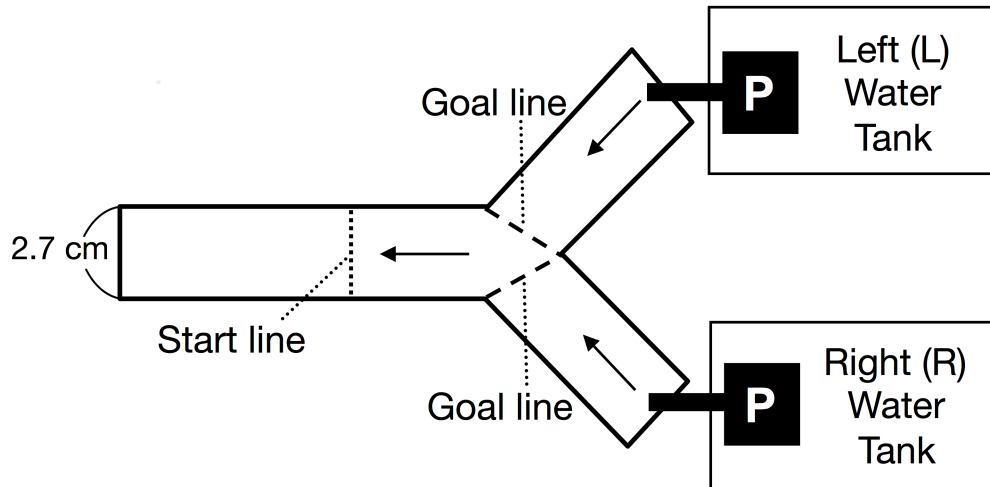
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356 Figure 1. Top views of the experimental channel. Arrows and P show the water flows and

357 water pump, respectively.

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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, $p < 0.05$).