

Plastic phenotypes of antennule shape in *Bosmina longirostris* controlled by physical stimuli from predators

Masaki Sakamoto¹

Institute of Mountain Science, Shinshu University, Kogandori 5-2-4, Suwa 392-0027 Japan

Kwang-Hyeon Chang

Center for Marine Environmental Studies, Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577 Japan

Takayuki Hanazato

Institute of Mountain Science, Shinshu University, Kogandori 5-2-4, Suwa 392-0027 Japan

Abstract

Three antennule morphotypes in the small cladoceran *Bosmina longirostris* have been considered to be hereditarily rigid phenotypes. However, we found that monoclonal populations of *Bosmina* collected from a Japanese lake were composed of individuals with all three antennule types when grown under laboratory conditions. This suggests that each of the morphotypes represents a phenotype of the species. A morphotype named “*pellucida*” observed in the monoclonal cultures has been considered to be effective in avoiding copepod predations. We therefore hypothesized that the morphotype is induced by factors from the predator, cyclopoid copepod. To test this hypothesis, a laboratory experiment was conducted in which the monoclonal individuals were exposed to the kairomones of the copepod *Acanthocyclops* with or without physical contacts with the predator. The morphotype was not altered when *Bosmina* was exposed to the predator kairomone alone, but to direct predation by the copepod. This suggests that the antennule type of *B. longirostris* is controlled by physical stimuli from the copepod. The individual death rate of *B. longirostris* resulting from a copepod strike is low, even though the population dynamics of the animals are often governed by copepod predation. This may have made the physical stimuli of attempted predations useful factors for the prey *Bosmina*.

Cyclomorphosis of cladocerans and rotifers is observed in many lakes and ponds (Agrawal 2001; Lass and Spaak 2003). Predator kairomones (chemicals released from predators; Brown et al. 1970) are the most prevailing cues for induction of these morphological changes (Lass and Spaak 2003). For example, *Daphnia* develops anti-predator morphologies such as high helmet, neck teeth, crest, elongated spine, and hard carapace, which help to reduce the prey mortality in environments with specific predators (Tollrian and Dodson 1999; Laforsch et al. 2004). However, it has been observed that the morphological changes in cladocerans induced by predator kairomones in the laboratory are not as extreme as those in the field (*Daphnia*: Havel and Dodson 1985; Hanazato and Ooi 1992; *Bosmina*: Sakamoto et al. 2006). This might indicate that laboratory cultures failed to reproduce all of the cyclomorphogenic conditions existing in the field (Jacobs 1987), or that researchers have not considered multigenerational cue effects on the prey animals (Agrawal et al. 1999; Tanner and Branstrator 2006).

Laboratory studies have revealed some factors other than kairomones that promote the development of protuberant morphological features in cladocerans. For

example, warmer temperature and water turbulence induce the formation of high helmets or crests in *Daphnia* even when predator kairomones are absent (Havel and Dodson 1985). Alarm substances released from crushed *Daphnia* have also been shown to act as proximate factors for the induction of these features (Stabell et al. 2003; Laforsch et al. 2006). These environmental conditions and chemical signals are considered to be effective in helping prey daphnids detect specific enemies, since they are identical to those associated with the abundance of predators in the field. High temperature is a feature of summer, a time when larvae of the invertebrate predator *Chaoborus* appear in abundance, and when both invertebrate and vertebrate predators are highly active (Tollrian and Dodson 1999). Small-scale water turbulence can be caused by the movements of swarming predators (Laforsch and Tollrian 2004; Tollrian and Laforsch 2006). Fluids released from crushed *Daphnia* (alarm substances) may notify conspecifics that they are in danger of predation (Laforsch et al. 2006). These are the factors to which daphnids are often exposed in the field. Therefore, it would be expected that daphnids are influenced by such factors simultaneously, and as a result would probably develop extreme morphologic features. However, information on such proximate factors is lacking for nondaphnid cladocerans.

Polymorphism is also observed in the small cladoceran *Bosmina*. In lakes and ponds, these animals are important prey items for fish and various invertebrate predators such as predacious copepods and cladocerans (Ślusarczyk 1997; Chang and Hanazato 2003a). In particular, their popula-

¹ Corresponding author (mslucky94@yahoo.co.jp).

Acknowledgments

We thank T. Nagata for his helpful comments on our work. This study was partly supported by Grants-in-Aid to T.H. (17201012) from Japan Society for the Promotion of Science.

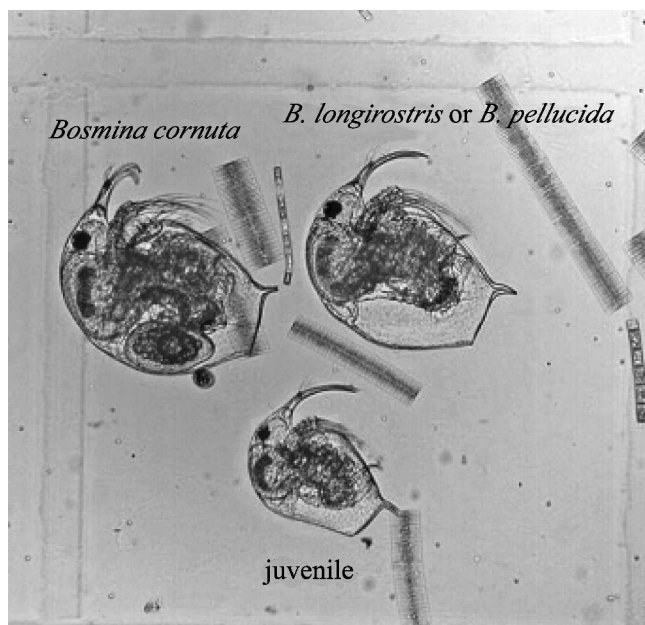


Figure 1. *Bosmina* species with different morphotypes appearing in Lake Suwa.

tion dynamics are often controlled more strongly by invertebrate than by vertebrate predators because of their small body size (Kerfoot 1977; McNaught et al. 2004). Bosminids show cyclomorphosis of the antennule and mucrone, whose lengths change in response to factors related to invertebrate predators (Kerfoot 1987; Kappes and Sinsch 2002a; Lagergren et al. 2002). However, the protuberant morphological features demonstrated in the laboratory, where bosminids have been exposed to predator kairomones, are not as extreme as those observed in the field, as is the case for *Daphnia* (*Bosmina fatalis*: Sakamoto et al. 2006). Another proximate factor that promotes the development of defensive morphotypes in *Bosmina* is known to be cold water. This is consistent with the water environment in early spring and fall, when bosminids are exposed to high abundances of predatory cyclopoid copepods (*Bosmina longirostris*: Kerfoot 1977; Kappes and Sinsch 2002a).

Physical cues as a factor inducing morphological changes have never been tested for *Bosmina*. In the present study, therefore, we hypothesized that some physical interactions with invertebrate predators induce the development of morphological defenses in *Bosmina*. To investigate this, we performed two laboratory experiments. The first one was conducted to reconsider the existing taxonomic problem in *B. longirostris*. In this species, animals with three antennule types without intermediate forms have been reported: Straight (*B. longirostris*), slightly curved (*Bosmina pellucida*), and hooked/bent (*Bosmina cornuta*), as shown in Fig. 1. Recently, antennule morphotype has been discussed as an indicator for defining cryptic species in *B. longirostris* (Kappes and Sinsch 2002b). However, we found that each of our laboratory-cultured clones, originally from a Japanese lake, was composed of individuals with different antennule types. Thus, the

antennule types of the *Bosmina* individuals cultured in the absence of predators were compared among monoclonal populations. In the second experiment, antennule types and the degree of development of protuberant morphology in *B. longirostris* were analyzed for the individuals exposed to copepod predation (physical stimuli and the predator kairomone or only to the kairomone). Then, the possibility that physical stimuli from the predators promote the development of the protuberant morphology was tested.

Materials and methods

Experiment 1. Clonal comparison—To obtain specimens of *B. longirostris*, we placed approximately 500 g of bottom mud from Lake Suwa, Japan (36°2'N, 138°5'E) containing cladoceran resting eggs in a 20-liter cylindrical polyethylene tank with 20 liters of aged tap water. The tank was kept at a constant temperature of 20°C ± 1°C under a light : dark (LD) regime of 16 : 8 LD. The green alga *Chlorella vulgaris* (Chlorella Industry) was added to the tank every second day to attain a density of 2.9 µg C mL⁻¹ as food for zooplankton. Three *Bosmina* individuals that appeared in the tank were collected and each individual was moved to a separate 200-mL beaker containing 200 mL of aged tap water and then kept under the same conditions as those for the hatching of resting eggs. The beakers received *Chlorella* (2.9 µg C mL⁻¹) every second day, and the *Bosmina* individuals were allowed to reproduce and establish populations. Then, more than 100 adult individuals were removed randomly from each monoclonal culture and their morphotypes (antennule types) were identified following the criterion shown in Fig. 2a. Because it was difficult to distinguish *longirostris* from *pellucida* using this criterion, we did not divide them into different morphotypes but placed them in the group *pellucida*. Animals with hooked antennules (typical morphotype of *B. cornuta*; Kappes and Sinsch 2002b) were identified as *cornuta*.

Experiment 2. Effects of the predator on bosminid morphology—A single clone of *B. longirostris* was used in this experiment. Preparation of the clone (collection and culture) was carried out in the same manner as described for experiment 1. The stock culture of cloned *Bosmina* in a 1-liter glass beaker received *Chlorella* (2.9 µg C mL⁻¹) every second day.

The copepod *Acanthocyclops vernalis* was used as the predator for *B. longirostris* because it is the dominant copepod coexisting with the prey *Bosmina* in Lake Suwa. *Acanthocyclops* individuals were isolated from plankton samples collected from the same lake in April 2005, and their body sizes were measured under a microscope to the nearest 0.02 mm. Adult females (body length > 1.2 mm) were maintained individually for 24 h at 20°C in polystyrene well chambers, each of which contained 2 mL of aged tap water without food until the start of the experiment.

At the beginning of the experiment, 12 groups each composed of randomly selected 50 individuals of *B. longirostris* (including both adults and juveniles) from the stock culture were prepared. The bosminids of each group

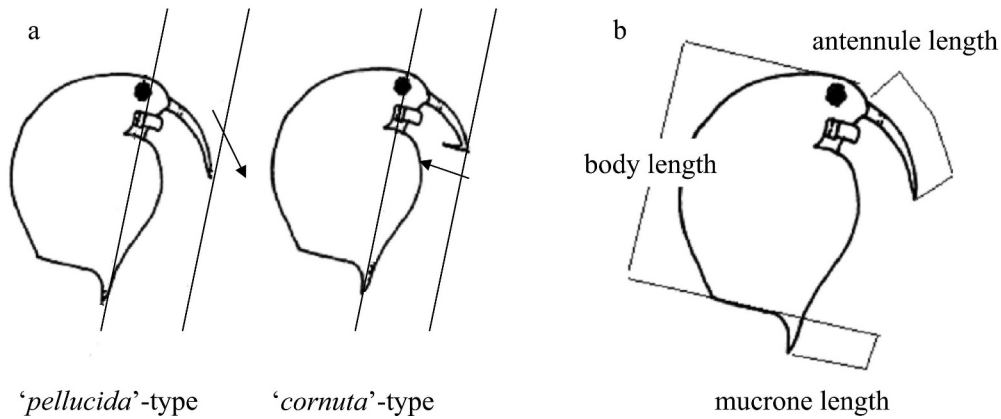


Figure 2. (a) Definitions of antennule type of *Bosmina* in the present study and (b) body measurements of animals. When the direction of the tip of the antennule is outside of the line connecting the compound eye and the tip of the mucrone, the animals were regarded as *pellucida* type and others were *cornuta* type.

were placed in a 1-liter glass beaker containing 1 liter of aged tap water and *Chlorella* ($2.9 \mu\text{g C mL}^{-1}$) as their food. Two cylindrical stainless steel nets (diameter 30 mm; height 80 mm; mesh size $44 \mu\text{m}$) were strung inside the beaker so that the bosminids examined were outside the nets (Fig. 3). The *Bosmina* individuals were exposed to each of the following four treatments. The first treatment was a control in which no *Acanthocyclops* were added to the beakers. In the second treatment (“isolation”), six *Acanthocyclops* individuals were placed in each stainless steel net. Thus, 12 predator individuals were present in each beaker, but were unable to have physical contact with *Bosmina*. The predators in the nets were given 10 individuals of *B. longirostris* from the stock culture as food every third day. In the third treatment (“no isolation”), 12 *Acanthocyclops* individuals were placed outside the nets, thus allowing them to have contact with, and feed on, the *Bosmina* individuals in the beakers. In these three treatments, 10 mg

of cetyl alcohol [$\text{CH}_3(\text{CH}_2)_{14}\text{CH}_2\text{OH}$] was gently dropped onto the water surface in each beaker as a surfactant to decrease the surface tension that otherwise might entrap the *Bosmina* individuals (Desmarais 1997). To confirm that the cetyl alcohol had no effect on the morphology of *Bosmina*, a fourth preparation (“no cetyl alcohol”) in which cetyl alcohol was omitted was made. All the treatments were conducted with three replications and *Chlorella* ($2.9 \mu\text{g C mL}^{-1}$) was added to each beaker every second day. The exposure of *Bosmina* to each treatment was terminated on the 13th day from the beginning of the experiment, and all the animals that had survived in each beaker were preserved with sugar-containing formalin at a final concentration of 4% (Haney and Hall 1973).

The bosminids were divided into two groups, adults (body length $> 300 \mu\text{m}$) and juveniles, and these were both counted. Antennule shapes of the adults were identified using the same method as that described for experiment 1

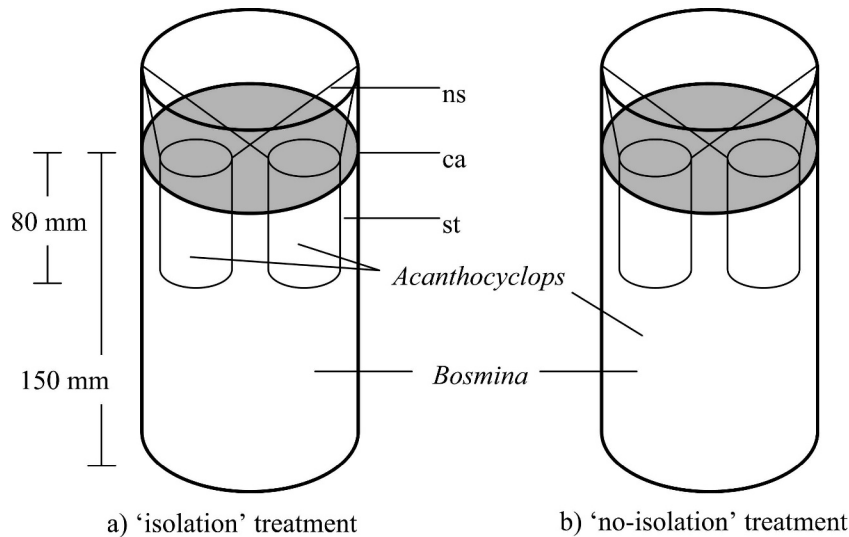


Figure 3. Schematic drawing of the experimental design. The copepods and *Bosmina* were (a) isolated by stainless steel nets or (b) allowed to come into contact with each other. Abbreviations: ns, nylon string; ca, cetyl alcohol; st, stainless steel net.

Table 1. Different proportions of the two antennule types observed in three *Bosmina longirostris* clones (adult females) cultured in the absence of predator in experiment 1.

Clone	Antennule type (ind.)		Total	<i>cornuta</i> (%)
	<i>cornuta</i>	<i>longirostris</i> or <i>pellucida</i>		
A	100	52	152	65.8
B	58	77	135	43.0
C	113	9	122	92.6

(Fig. 2a). In addition, each individual was measured for body, antennule, and mucrone lengths under a binocular microscope to the nearest 0.01 mm (Fig. 2b). Differences in the morphotype composition among the four treatments were tested statistically with the Bonferroni/Dunn post hoc test (after the analysis with analysis of variance [ANOVA]) for the values of relative abundance of *cornuta* (number of *cornuta*:total adult females) after arcsine transformation using StatView version 5 (SAS Institute). Quantitative differences in morphological measurements between the four treatments were also tested by nested ANOVA to assess the effects of the treatments.

Results

Experiment 1. Clonal comparison—More than one antennule type was observed in all of the three monoclonal populations of *B. longirostris* (Table 1). The percentage prevalence of *cornuta*-type individuals (with hooked antennules) varied depending on the clone. About half of the adults showed the *cornuta*-type morphotype and the others had straight or slightly curved (*pellucida*-type) antennules in clones A and B. A markedly higher percentage of *cornuta* was recorded in clone C, in which 93% of adults had hooked antennules.

Experiment 2. Effects of the predator on bosminid morphology—The numbers of *Bosmina* individuals in the no cetyl alcohol and *Acanthocyclops* treatments (isolation and no isolation) at the end of the experiment were smaller than in the control (Table 2). In the control, all the juveniles had slightly curved antennules, whereas most adult individuals (89%) had hooked antennules (*cornuta*) and the others had straight or slightly curved antennules (*pellucida*). The percentages of *cornuta* in the no cetyl alcohol treatment and the isolation treatment were similar

to that in the control. As a result, the number of individuals of each morphotype was close to the expected individual number (E_N) of each morphotype (Table 2), which was calculated as $E_N = N_T P_M$, where N_T is the total number of individuals in the treatment at the end of the experiment, and P_M is the proportion of individuals showing each morphotype to the total individuals in the control. In contrast, the percentage of *cornuta* individuals in the no-isolation treatment was significantly lower than that in the control (ANOVA, $p < 0.01$; Bonferroni/Dunn post hoc test, $p < 0.05$), and the absolute number of *pellucida* individuals was obviously higher than the expected value.

No significant differences in the lengths of the body, antennule, and mucrone of the animals showing each morphotype were detected among the treatments in both juveniles and adults ($p > 0.05$ with nested ANOVA), although the antennule tended to be longer in the isolation and no-isolation treatments than in the other treatments within the *pellucida* morphotype, and the mucrone tended to be longer in the no-isolation treatment for both morphotypes (Fig. 4). The mean antennule length for *pellucida* in the predator treatments (isolation and no isolation) and mucrone length in the no-isolation treatment for both morphotypes were similar to those for animals present in the field in Lake Suwa (Chang and Hanazato 2003a).

Discussion

The *B. longirostris* individuals in monoclonal populations had varied antennule shapes (Tables 1, 2). This clearly shows that the different morphotypes *longirostris*, *pellucida*, and *cornuta* are different phenotypes within a species but not hereditarily rigid. The *B. longirostris* clones used in the present experiments were different from clones of the same species reported by Kappes and Sinsch (2002a), in which antennule shapes were invariable phenotypes. The difference in results between the two studies may be explainable by “local adaptation” (Kawecki and Ebert 2004) or simple clonal differences in *Bosmina* (Table 1). Genotype-dependent flexibilities in the phenotypic plasticity of Cladocera have been reported mainly for the genus *Daphnia* (Lüning 1994, Boersma et al. 1998).

The total number of *Bosmina* individuals at the end of the experiment was higher in the control than in the no cetyl alcohol treatment (Table 2). The lower number of individuals resulting from the latter treatment might have

Table 2. Mean numbers of individuals with different antennule types (mean \pm SE) in a beaker in experiment 2. Values in parentheses are the expected values (see explanation in text).

Treatment	Adult	Antennule type in adult			Juvenile
		<i>cornuta</i>	<i>pellucida</i>	<i>cornuta</i> (%)	
Control	40.3	36.0 \pm 6.7	4.33 \pm 0.3	88.7 a*	36.7
No cetyl alcohol	15.3	11.7 \pm 2.6 (13.7)	3.67 \pm 1.5 (1.65)	78.1 a	13.7
Isolation	17.7	15.3 \pm 3.9 (15.8)	2.33 \pm 0.3 (1.90)	86.2 a	12.0
No isolation	14	5.0 \pm 2.9 (12.5)	9.0 \pm 2.9 (1.50)	25.8 b	6.67

* Means denoted by the same letter are not significantly different from each other with Bonferroni/Dunn post hoc test ($p > 0.05$).

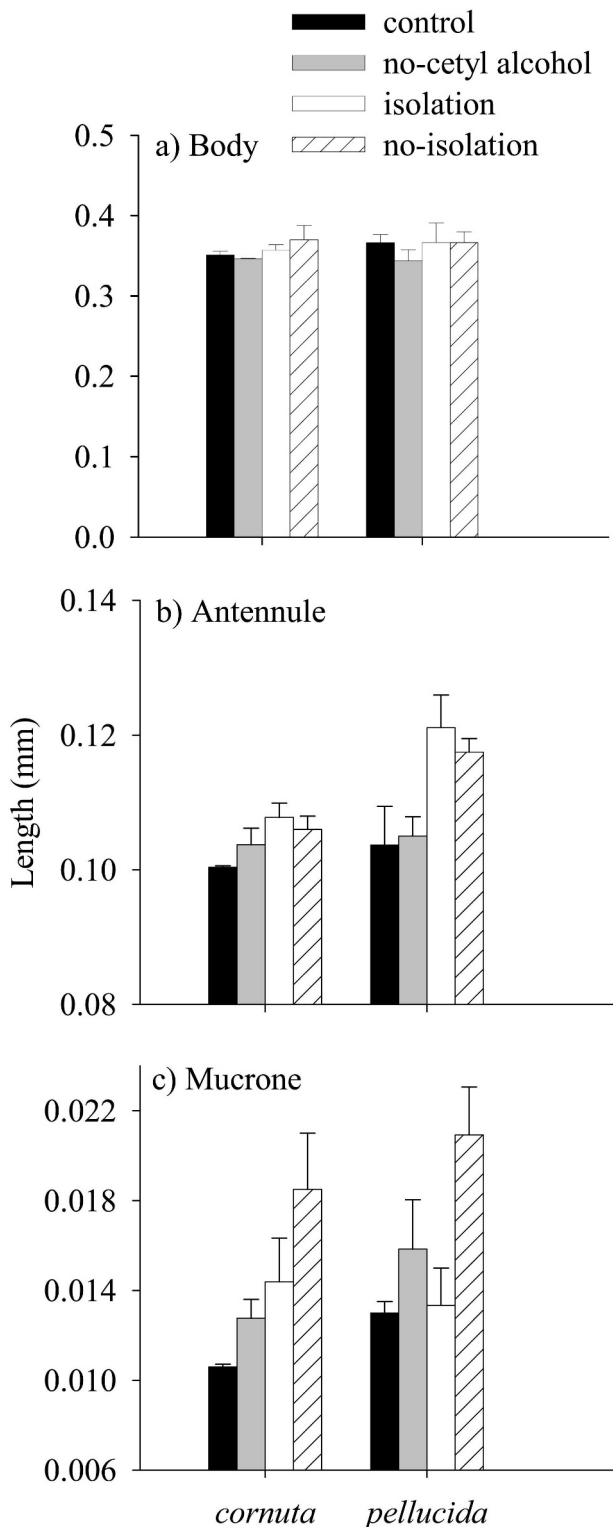


Figure 4. Length (mean \pm SE) of (a) the body, (b) the antennule, and (c) the mucrone of *B. longirostris* with each antennule shape at the end of the experiment.

been due to entrapment at the water surface. This indicated that the cetyl alcohol treatment was effective in reducing mortality of *Bosmina* caused by entrapment, as was the case for *Daphnia* (Desmarais 1997). A marked decline in the

number of animals was also observed in the *Acanthocyclops* treatments (isolation and no isolation), even though the water had been treated with cetyl alcohol. This might have been due to predation by *Acanthocyclops* in the no-isolation treatment and some other unknown factors in the isolation treatment. A possible factor would be the negative impact of the predator kairomone, which is known to suppress the individual growth of cladocerans, especially *Daphnia*, which lose energy in defensive response to predators (Tollrian and Dodson 1999). In addition, the negative impact might be promoted by the not-very-favorable food condition with *Chlorella* as food in the present experiment, where *Bosmina* showed a slower population growth in comparison with the result of a study by Urabe (1991), who used mixed algae comprised mainly of *Scenedesmus*. Actually, *B. longirostris* fed with *Chlorella* in our separate culture experiment showed lower fecundity than the *Bosmina* did in Urabe's experiment even though the algal biomass prepared for the animals was nearly the same in the two experiments.

The results of experiment 2 indicated that the proportion of antennule types was constant (ca. 80% of individuals showing the *cornuta*-type morphotype) in most of the treatments (control, no cetyl alcohol, and isolation). However, this was not the case for the no-isolation treatment, where predators were allowed to physically attack the prey *Bosmina* and the ratio of *cornuta* was significantly lower than that in the other treatments (Table 2). The difference in the relative abundance of *cornuta*-type individuals must therefore have been due to the bosminids having direct contact with the copepods. Cyclopoid copepods can consume bosminids with shorter antennules and mucrones more efficiently than those with longer appendages (Kerfoot 1977, 1987). In our data, the *cornuta* morphotype tended, but not significantly, to have shorter antennules than the *pellucida* morphotype in the no-isolation treatment (Fig. 4a). This coincides with the findings by Kappes and Sinsch (2002a, b), who reported that *B. longirostris* with the *cornuta* morphotype have shorter appendages than the animals with the *pellucida* morphotype. Therefore, it seems most likely that *cornuta* is more vulnerable to copepod predation than *pellucida*, as indicated by Kappes and Sinsch (2002a). Thus, the decline in the percentage and absolute number of *cornuta* individuals in the no-isolation treatment can be well explained by selective predation. Surprisingly, however, the absolute number of *pellucida* individuals was much higher than the expected value (Table 2). This means that the number of *pellucida*-type individuals increased during treatment.

The reason why *pellucida* individual increased in the no-isolation treatment may be explained as follows. There was competition between the *cornuta* and *pellucida* individuals, and their relative abundance was determined by the competitive pressure. The pressure was reduced by predation of copepods, which decreased total number of bosminids, and as a result, the *pellucida* (defensive morphotype) individuals increased while the *cornuta* (nondefensive morphotype) decreased by selective predation by copepods.

However, this might not be the event that occurred in the isolation treatment, because the competitive pressure seemed not to be controlled by the predators. This was inferred from the phenomenon that the total number of *Bosmina* in each experimental chamber decreased not only in the no-isolation treatment but also in the isolation treatment. The decline in the individual number might be due to entrapment to the water surface or other unknown factors. Thus, it could be concluded that in the no-isolation treatment, some animals altered their antennule shape from the *cornuta* type to the *pellucida* one.

The results of the present study and that of Kappes and Sinsch (2002a) suggest that the *pellucida* morphotype is more effective in avoiding cyclopoid copepod predation than the *cornuta* morphotype. We observed that all the juvenile bosminids had the *pellucida* morphotype (appendage lengths were not affected by the treatments, $p > 0.05$ with nested ANOVA), but that this mostly changed to the *cornuta* type during growth in the control and isolation treatments, where the animals were not attacked by predators. This phenomenon may indicate that the juvenile life stage is the one most vulnerable to copepod predation (Kerfoot 1977; Chang and Hanazato 2005), thus explaining why juveniles always possess the defensive morphotype irrespective of presence/absence of predators. Loss of the *pellucida* morphotype in the adult stages suggests that retaining the relatively straight and long antennules carries some disadvantages in the absence of predators. Lagergren et al. (1997) estimated the cost associated with the extreme morphotype (high carapace and extremely long antennules) of *Eubosmina coregoni gibbera* in comparison with the typical morphotype (low body height and short antennules) of *Eubosmina longispina longispina*, and concluded that animals showing the extreme form had to work at least 12% harder than those with the typical form to swim at the same speed because of the increased viscous drag. This is also likely the case for *B. longirostris* with the *pellucida* morphotype, and probably explains why most adults lose the defensive morphotype in the absence of predators.

The number of *pellucida*-type individuals increased in the no-isolation treatment but did not in the isolation treatment. This suggests that the change in antennule shape from *cornuta* to *pellucida* was not induced by chemical cues (predator kairomone), but rather by physical stimulation (turbulence or attack) by *Acanthocyclops*. It is possible that substances released from consumed or injured *Bosmina* act as alarm signals (*Daphnia*: Laforsch et al. 2006). However, this may be denied by the fact that no morphological change was induced in the isolation treatment, where *Bosmina* were constantly introduced into the inside of nets as food for *Acanthocyclops* and should be ingested or damaged by the predators. Therefore, we hypothesize that physical stimulation by invertebrate predators acts as an induction factor for morphological changes in *B. longirostris*. To our knowledge, this is the first study demonstrating that physical stimulation by predators is a proximate factor inducing the development of defensive morphologies in zooplankton species excepting *Daphnia* (Tollrian and Laforsch 2006).

The induction of defense strategies by mechanical damage resulting from predation has been reported in

both terrestrial plants and animals (Agrawal et al. 1999; Agrawal 2001). For example, high levels of proteinase inhibitors (high trypsin inhibitor:soluble plant protein ratio) in the grey alder (*Alnus incana*) are induced by grazing of the leaf beetle *Galerucella lineola*, and act as a chemical defense that restricts the growth of the herbivore (Seldal et al. 1994). Harvell (1984) demonstrated that the marine bryozoan *Membranipora membranacea* produced defensive spines in response to stimulation by predatory nudibranchs (*Doridella steinbergae* and *Onchidoris muricata*). Such physical damage acts as a prompt warning signal to prey organisms, which then adopt appropriate countermeasures. On the other hand, chemical stimuli (predator kairomones), which are well known cues used in prey-predator interactions in plankton communities, help prey individuals to take preventive measures as they are able to detect the presence of predators before they are physically attacked (Hanazato 1994). Our studies have confirmed that the small cladoceran *Bosmina* develops defensive morphotypes in response to physical (this study), not only to chemical (Sakamoto et al. 2006), stimuli originating from predators.

In the present study, *Bosmina* tended (especially in *pellucida*, but not significantly) to have longer antennules in the isolation treatment, where it was exposed to the copepod kairomone (Fig. 4a). However, the change in antennule morphotype was not induced by the kairomone but by physical contact with the predator (Table 2). These facts suggest that exposure to the predator kairomone alone is insufficient to trigger the development of extreme morphologies, and that mechanical cue(s) from predators act as an additional proximate factor for induction of these features in *Bosmina*. In the present experiment, we did not expose *B. longirostris* to cold water, which is known to be a factor enhancing the development of protuberant morphotypes of this species (Kappes and Sinsch 2002a). Thus, induction of more extreme morphologies would likely be observed if the animals were exposed to such environmental factors simultaneously with physical and chemical cues.

The validity of the morphological defenses of prey animals in response to physical stimuli may depend on the feeding behavior of the predators. If the prey organisms have high death rates as a result of a predator strike, then physically induced morphological defenses may not evolve. Kerfoot (1977) tested the predation efficiency of two cyclopoid copepod species for *B. longirostris*, and confirmed that the percentage of first-instar individuals injured was 2% after encounters with *Cyclops bicuspidatus* and 21% with *Cyclops vernalis*. The rates of injury decreased with growth of the bosminids. Chang and Hanazato (2003b) reported that the capture efficiency and the probability of successful ingestion of *B. longirostris* by the cyclopoid copepod *Mesocyclops* were less than 20% during 1.5 h when two prey and a predator were placed in a 1-mL chamber. These results suggest that prey *Bosmina* are not captured easily by the copepods even if attacked frequently, and thus the physical stimuli provided by the predators are useful signals that enable the prey animals to predict increased mortality due to predation.

References

- AGRAWAL, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* **294**: 321–326.
- , C. LAFORSCH, AND R. TOLLRIAN. 1999. Transgenerational induction of defences in animals and plants. *Nature* **40**: 60–63.
- BOERSMA, M., P. SPAAK, AND L. DEMEESTER. 1998. Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: The uncoupling of responses. *Am. Nat.* **152**: 237–248.
- BROWN, W. L., JR., T. EISNER, AND W. H. WHITTAKER. 1970. Allomones and kairomones: Transspecific chemical messengers. *BioScience* **20**: 21–22.
- CHANG, K. H., AND T. HANAZATO. 2003a. Seasonal and reciprocal succession and cyclomorphosis of two *Bosmina* species (Cladocera, Crustacea) co-existing in a lake: Their relationship with invertebrate predators. *J. Plankton Res.* **25**: 141–150.
- , AND ———. 2003b. Vulnerability of cladoceran species to predation by the copepod *Mesocyclops leuckarti*: Laboratory observations on the behavioural interactions between predator and prey. *Freshw. Biol.* **48**: 476–484.
- , AND ———. 2005. Impact of selective predation by *Mesocyclops pehpeiensis* on a zooplankton community: Experimental analysis using mesocosms. *Ecol. Res.* **20**: 736–732.
- DESMARIS, K. H. 1997. Keeping *Daphnia* out of the surface film with cetyl alcohol. *J. Plankton Res.* **19**: 149–154.
- HANAZATO, T. 1994. Kairomone-mediated predator–prey relationship in lake zooplankton communities. *Jap. J. Ecol.* **44**: 61–77. [In Japanese.]
- , AND T. OOI. 1992. Morphological responses of *Daphnia ambigua* to different concentrations of a chemical extract from *Chaoborus flavicans*. *Freshw. Biol.* **27**: 379–385.
- HANEY, J. F., AND D. J. HALL. 1973. Sugar-coated *Daphnia*: A preservation technique for Cladocera. *Limnol. Oceanogr.* **18**: 331–333.
- HARVELL, C. D. 1984. Predator-induced defense in a marine bryozoan. *Science* **224**: 1357–1359.
- HAVEL, J., AND S. I. DODSON. 1985. Environmental cues for cyclomorphosis in *Daphnia retrocurva* Forbes. *Freshw. Biol.* **15**: 469–478.
- JACOBS, J. 1987. Cyclomorphosis in *Daphnia*, p. 325–352. In R. H. Peters and R. de Bernardi [eds.], *Daphnia*. Mem. Ist. Ital. Idrobiol.
- KAPPES, H., AND U. SINSCH. 2002a. Temperature- and predator-induced phenotypic plasticity in *Bosmina cornuta* and *B. pellicida* (Crustacea: Cladocera). *Freshw. Biol.* **47**: 1944–1955.
- , AND ———. 2002b. Morphological variation in *Bosmina longirostris* (O.F. Müller,) (Crustacea: Cladocera): Consequence of cyclomorphosis or indication of cryptic species? *J. Zool. Syst. Evol. Res.* **40**: 113–122.
- KAWECKI, T. J., AND D. EBERT. 2004. Conceptual issues in local adaptation. *Ecol. Lett.* **7**: 1225–1241.
- KERFOOT, W. C. 1977. Implications of copepod predation. *Limnol. Oceanogr.* **22**: 316–325.
- . 1987. Translocation experiment: *Bosmina* responses to copepod predation. *Ecology* **68**: 596–610.
- LAFORSCH, C., L. BECCARA, AND R. TOLLRIAN. 2006. Inducible defenses: The relevance of chemical alarm cues in *Daphnia*. *Limnol. Oceanogr.* **51**: 1466–1472.
- , W. NGWA, W. GRILL, AND R. TOLLRIAN. 2004. An acoustic microscopy technique reveals hidden morphological defenses in *Daphnia*. *Proc. Natl. Acad. Sci. USA* **101**: 15911–15914.
- , AND R. TOLLRIAN. 2004. Extreme helmet formation in *Daphnia cucullata* induced by small-scale turbulence. *J. Plankton Res.* **26**: 81–87.
- LAGERGREN, R., M. HELLSTEN, AND J. A. E. STENSON. 1997. Increased drag, and thus lower speed: A cost for morphological defence in *Bosmina (Eubosmina)* (Crustacea: Cladocera). *Funct. Ecol.* **11**: 484–488.
- , J. E. SVENSSON, AND N. LUNDQVIST. 2002. Clutch size variation and morphology in a cyclomorphic *Bosmina* population. *J. Plankton Res.* **24**: 653–659.
- LASS, S., AND P. SPAAK. 2003. Chemically induced anti-predator defences in plankton: A review. *Hydrobiologia* **491**: 221–239.
- LÜNING, J. 1994. Anti-predator defenses in *Daphnia*—are life-history changes always linked to induced neck spines? *Oikos* **69**: 427–436.
- MCCAUGHT, A. S., R. L. KIESLING, AND A. GHADOUANI. 2004. Changes to zooplankton community structure following colonization of a small lake by *Leptodora kindtii*. *Limnol. Oceanogr.* **49**: 1239–1249.
- SAKAMOTO, M., K. H. CHANG, AND T. HANAZATO. 2006. Inhibition of development of anti-predator morphology in the small cladoceran *Bosmina* by an insecticide: Impact of an anthropogenic chemical on prey–predator interactions. *Freshw. Biol.* **51**: 1974–1983.
- SELDAL, T., E. DYBWAD, K. J. ANDERSEN, AND G. HÖGSTEDT. 1994. Wound-induced proteinase inhibitors in grey alder (*Alnus incana*): A defence mechanism against attacking insects. *Oikos* **71**: 239–245.
- ŚLUSARCZYK, M. 1997. Impact of fish predation on a small-bodied cladoceran: Limitation or stimulation? *Hydrobiologia* **342/343**: 215–221.
- STABELL, O. B., F. OGBEBO, AND R. PRIMICERIO. 2003. Inducible defences in *Daphnia* depend on latent alarm signals from conspecific prey activated in predators. *Chem. Senses* **28**: 141–153.
- TANNER, C. J., AND D. K. BRANSTRATOR. 2006. Generational and dual-species exposures to invertebrate predators influence relative head size in *Daphnia mendotae*. *J. Plankton Res.* **28**: 793–802.
- TOLLRIAN, R., AND S. I. DODSON. 1999. Inducible defenses in Cladocera: Constrains, costs, and multipredator environments, p. 177–202. In R. Tollrian and C. D. Harvell [eds.], *The ecology and evolution of inducible defenses*. Princeton Univ. Press.
- , AND C. LAFORSCH. 2006. Linking predator kairomones and turbulence: Synergistic effects and ultimate reasons for phenotypic plasticity in *Daphnia cucullata*. *Arch. Hydrobiol.* **167**: 135–146.
- URABE, J. 1991. Effect of food concentration on growth, reproduction and survivorship of *Bosmina longirostris* (Cladocera): An experimental study. *Freshw. Biol.* **25**: 1–8.

Received: 28 December 2006

Accepted: 3 May 2007

Amended: 15 May 2007