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Differential Sensitivity of a Predacious Cladoceran (*Leptodora*) and Its Prey (the Cladoceran *Bosmina*) to the Insecticide Carbaryl: Results of Acute Toxicity Tests

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Anthropogenic toxic chemicals contaminate various water bodies and may affect aquatic organic communities. To assess the impacts of chemicals on communities, laboratories perform toxicity tests, using the freshwater herbivorous zooplankton *Daphnia magna* and *D. pulex* as standard organisms (OECD 1984), since they are among the most sensitive animals to toxic chemicals in water, and underpin many lake ecosystems (Hanazato 2001). However, this is not the case for shallow and eutrophic lakes with abundant fish, where the herbivorous zooplankton community is often dominated by small cladocerans such as the genus *Bosmina*, not by large *Daphnia*.

Bosmina is a widespread and abundant cladoceran. The bosminids are important food organisms for fish and various invertebrate predators such as cyclopoid copepods and predacious cladocerans. In particular, the predacious cladoceran *Leptodora* is a common predator in eutrophic fish-abundant lakes, and has a significant impact on zooplankton communities (McNaught et al. 2004). Bosminids are favored prey of *Leptodora*, and their population dynamics are often controlled by predation by *Leptodora* (Chang and Hanazato 2004). At the same time, *Leptodora* itself is a preferred prey item for fish (Bramstrator and Holl 2000). Thus, *Bosmina* and their predator *Leptodora* play important roles in the energy flow from primary producer to the top predators (fish) in eutrophic lakes.

Despite the important role of bosminids and *Leptodora* in freshwater ecosystems, information on their sensitivities to toxic chemicals is still insufficient. The sensitivities of the Bosminidae to insecticides have often been estimated from the results of community-level experiments using mesocosms (Havens 1994; Hanazato 2001), from which *Bosmina longirostris* and *B. fatalis* have been considered to be less sensitive to the insecticide carbaryl (a carbamate insecticide, 1-naphthyl-N-methylcarbamate) than *Daphnia galeata* and *D. ambigua*. In contrast, the acute toxicity tests of *B. longirostris* with arsenate and DDT showed that *B. longirostris* was more sensitive to the chemicals than *D. pulex* was (Passino and Novak 1984). This indicates the possibility that the sensitivities of *Bosmina* to insecticides estimated from community-level experiments differ from those from individual-level tests. However, few tests of *Leptodora* with

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insecticides have been conducted. Thus, to evaluate the impacts of pesticides on freshwater ecosystems, it is necessary to conduct precise individual-level toxicity tests with these animals.

In the present study, we performed toxicity tests on two *Bosmina* species (*B. longirostris* and *B. fatalis*) and *Leptodora kindtii* with the insecticide carbaryl. *B. longirostris* and *L. kindtii* are cosmopolitan species, while *B. fatalis* is restricted to east and southeast Asia. Carbaryl is commonly used on various agricultural and horticultural fields in Japan, and its toxicity to various *Daphnia* species has been well tested (Hanazato 2001).

MATERIALS AND METHODS

To obtain specimens of *B. longirostris* and *B. fatalis* (Fig. 1) as test animals, we placed approximately 500 g of bottom mud from the eutrophic Lake Suwa, Japan (36°2′N, 138°5′E), containing resting eggs of these cladocerans, in a 20-L cylindrical polyethylene tank (diameter, 30 cm; height, 31 cm) with 20-L of aged tap water. The tank was kept at a constant temperature of 20 ± 1 °C and a light-dark regime of 16-h light and 8-h darkness. The green alga *Chlorella* (Chlorella Industry Co. Ltd, Fukuoka, Japan; 1×10^5 cells/mL) was introduced into the tank as food for zooplankton every second day. Each *Bosmina* individual that appeared in the tank was collected and moved to a 1-L beaker containing 1 L of aged tap water and kept under the same conditions. Each beaker received *Chlorella* (1×10^5 cells/mL) every second day, and each individual established a population. Individuals of single clone were used for tests.

Carbaryl of >99% purity was purchased from Wako Pure Chemical Industries Ltd. Japan. A stock solution (1000 mg/L) was prepared by dissolving 10 mg in 99% ethanol to a final volume of 10 mL.

We followed OECD guidelines for testing chemicals (OECD 1984), with slight modifications, for 24-h bioassays. Test organisms were adult females of both species. The stock solution of carbaryl was diluted with aged tap water to seven concentrations (1.22, 1.95, 3.12, 5.00, 8.00, 12.80, and 24.48 μg/L), and a solvent (ethanol) control was prepared as well. Ten glass bottles (12 mL) were filled with each test solution. One animal was put into each bottle, and its exposure to the chemical was started. The mouth of each bottle was covered with a cover-glass to shut out the air from the test water so as to avoid trapping the animals at the water surface. Even so, one or two individuals were usually trapped at each chemical concentration, and so were omitted in determining mortality. The same temperature and light regime as above were used. At 24 hours after the start of the exposure, mortality was analyzed, and 24-h LC_{50} values with 95% confidence intervals were determined by Probit analysis with the program EcoTox-Statics Version2.5 (Oita Univ., Japan).

Leptodora kindtii were isolated from plankton samples collected from Lake Suwa

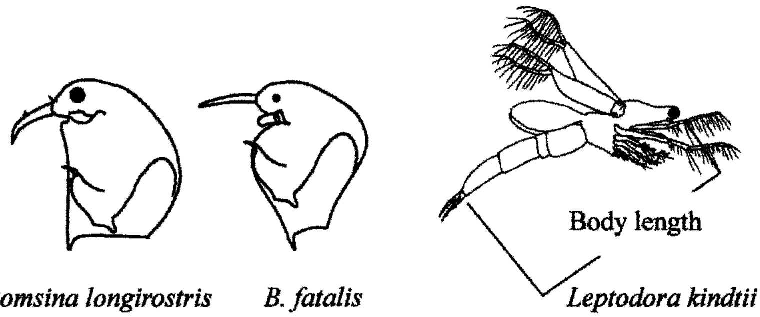

Figure 1. Morphology of animals used in the toxicity tests, and body length measurement of *Leptodora.*

in September 2004, and their body sizes were measured under a microscope to the nearest 0.02 mm (Fig. 1). The animals were divided into two size classes, small juveniles (mean ± SE: 2.003 ± 0.035 mm) and large animals (3.451 ± 0.061 mm). In the latter size class, some animals had brood chambers. Thus, the class included animals from large juveniles to middle-sized adults. They were maintained for more than 12 h in a 1-L beaker with aged tap water at a density of 10 inds./L to acclimatize them to the laboratory conditions. They were fed with natural zooplankton, including *Bosmina*, collected from the same lake.
Bioassays were performed as for *Bosmina*. The test was conducted twice for each size class. Mortality was determined at 12 and 24 h after the start of the chemical exposure, and the 12- and 24-h LC_{50} values with 95% confidence intervals were calculated as above.

RESULTS AND DISCUSSION

The 24-h LC_{50} value of *B. longirostris* was 8.6 µg/L (Table 1). This is similar to the 4-day value (7 µg/L) obtained from a mesocosm experiment (Havens 1994). The 24-h LC_{50} value of *B. fatalis* was 4.1 µg/L, indicating that this species is twice as sensitive to carbaryl. These two species often coexist and show a reciprocal seasonal succession pattern in lakes: *B. longirostris* dominates the zooplankton community in spring and is replaced by *B. fatalis* in summer (Hanazato et al. 1984; Chang and Hanazato 2003). We previously reported that cyanobacterial blooms and predation by *Leptodora* and cyclopoid copepods induce the seasonal succession (Hanazato and Yasuno 1987; Chang and Hanazato 2003). Now we propose insecticide contamination as another possible cause, because Japanese lakes are often contaminated with insecticide runoff from rice fields in spring (Shiraishi et al. 1988), when *B. longirostris*, which is more tolerant to insecticides, becomes dominant in lakes.

Sensitivity to pesticides may differ among cladoceran species. However, data from toxicity tests on most cladocerans except the Daphnidae are scarce, since daphnids are easier to breed than other species. Differences in sensitivity to pesticides among the species have been estimated from the results of mesocosm

Table 1. Acute toxicity (12- and 24-h LC_{50} values with 95% confidence limits in brackets) of carbaryl to *Bosmina longirostris*, *B. fatalis*, and their predator *Leptodora kindtii*. The test for *Leptodora* was carried out twice (1st, 2nd).

Organism		LC_{50} values in µg after	
		12 hours	24 hours
Prey			
Bosmina longirostris		not observed	8.597 [6.112, 13.246]
Bosmina fatalis		not observed	4.075 [1.971, 6.476]
Predator			
Leptodora kindtii (large)	1st	20*	3.605 [0.749, 6.367]
	2nd	23*	1.998 [1.148, 31.026]
	mean	22*	3.477 [1.225, 7.467]

* Approximate value; 95% confidence limits could not be calculated.

experiments (Havens 1994; Hanazato 2001). In an experiment in which carbaryl was added to mesocosm tanks housing a zooplankton community established from resting stages of zooplankton from the bottom mud of Lake Kasumigaura (Japan), larger cladoceran species tended to be more sensitive than smaller ones (*Daphnia* > *Moina*, *Diaphanosoma* > *Bosmina*) (Hanazato 1991). The same trend was found in another experiment, in which carbaryl was added to enclosures set up in a Lake in the USA (Havens 1994). Hanazato and Kasai (1995) reported similar results in a mesocosm experiment with the insecticide fenthion. Thus, it might be concluded that *Bosmina* are, in general, more tolerant to insecticides than other cladoceran species. However, the carbaryl concentration that affected the population of *B. fatalis* differed between the mesocosm experiments and the present toxicity test: in the mesocosms, the *B. fatalis* population was not affected by carbaryl at 100 µg/L (Hanazato 1991), which was far higher than the 24-h LC_{50} value (4.1 µg/L) obtained from the toxicity test. The 24-h LC_{50} values for *Bosmina* were also lower than the 48-h LC_{50} values of about 10 µg/L carbaryl for *Daphnia magna* (Hatakeyama and Sugaya 1989) and 6.87 µg/L for *D. pulex* (Hanazato and Hirokawa 2001), indicating that *Bosmina* is more sensitive to carbaryl than *Daphnia*. Equally, Passino and Novak (1984) have reported that *B. longirostris* is more sensitive to DDT than *D. pulex*. These results are inconsistent with the results of mesocosm experiments.

To our knowledge, there is no report on the sensitivity of *Leptodora* to insecticides. This is probably because *Leptodora* is too delicate to deal with in laboratory toxicity tests. In the present study, although we conducted the tests twice, most of the small juveniles died within the first 12 h in all treatments, including the control. Thus, their LC_{50} value was not determined. In contrast, most of the larger individuals survived in the control treatment, and we could obtain their mean 12- and 24-h LC_{50} values, which were approximately 22 and 3.48 µg/L, respectively (Table 1).

The results of this study show that sensitivity to carbaryl differs between the predator *Leptodora* and the prey *Bosmina*, the former being more sensitive. This suggests, therefore, that survivorship of *Bosmina* might increase in the presence of *Leptodora* when lakes are contaminated with insecticides.

Several researchers have studied the influence of insecticides on prey-predator interactions. For example, the freakish behavior of *Daphnia* and *Palaemonetes* (grass shrimp) and increased swimming speed of the rotiferan *Brachionus* induced by insecticides make them more attractive to visually oriented predator fish or increase their encounter rates with invertebrate predators, resulting in increased predation (Farr 1997; Dodson et al. 1995; Preston et al. 1999). In these cases, the prey animals are more sensitive to the insecticides than their predators, and behavioral changes induced by the chemicals in the prey elevate the predators' ingestion rates. In contrast, the present study shows a higher sensitivity of a predator to an insecticide than of their prey, suggesting the presence of a new type of pesticide impact on prey-predator interactions.

In the situation where predators are more sensitive to insecticides than their prey, the biomass of the prey is not carried efficiently to the animals at higher trophic levels, and thus energy transfer efficiency from primary producers to top predators is reduced.

This analysis of the sensitivities to an insecticide of two cladoceran herbivores and a carnivore, which have rarely been studied, reveals important information for evaluating the impact of insecticides on prey-predator interactions and on ecosystem functioning in lakes. However, the information is still insufficient. Toxicity tests performed for various aquatic organisms at different trophic levels will allow a more accurate evaluation of the impacts of anthropogenic chemicals on aquatic ecosystems.

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