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Dietary Program for Rearing the Larvae of a Diving Beetle, *Dytiscus sharpi* (Wehncke), in the Laboratory (Coleoptera: Dytiscidae)

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Abstract

For the conservation of the diving beetle *Dytiscus sharpi* (Wehncke) (Coleoptera: Dytiscidae), which is included on the Red Data List of Japan, it is critical to understand its ecological background. In the present study, we focused on its feeding behavior and nutritional needs under laboratory breeding conditions. First, we made a list of the possible candidates of prey in the same habitats where we caught *D. sharpi*. We found that the tadpoles of *Rana ornativentris* (Werner) were the major species present from March to April, when the beetle larvae appeared. Second, under our laboratory conditions, we investigated the size preference of beetle larvae preying on *R. ornativentris* tadpoles. We found a significant positive correlation between the developing stage of the larvae and the preferred prey size, *i.e.*, the first and third instars preferred smaller and larger prey, respectively, but second instars did not show any size preference. The size of full–grown adult beetles was almost the same as that of wild insects found in the field, indicating that *R. ornativentris* tadpoles provide almost complete nutrition for larval growth. Finally, we investigated how the size and number of *R. ornativentris* tadpoles were correlated with the developing stage of beetle larvae. We suggest that it is crucial for *Dytiscus* larvae to have access to tadpoles of the proper size and amounts, depending on their growth stage.

The population sizes of Japanese diving beetles (Coleoptera: Dytiscidae) have decreased and many species are included on the Red Data List of Japan, a list of threatened endangered species in Japan (Ministry of the Environment, Government of Japan 2000). Freshwater species and their habitats are increasingly threatened worldwide as well (Allan and Flecker 1993; Saunders *et al.* 2002). Biological factors affecting these endangered species, including a decrease in suitable aquatic habitats, urbanization, and water pollution especially from pesticide applications, have been investigated (Ministry of the Environment, Government of Japan 2000).

In the case of predacious insects, population size is often limited by food resources (Lenski 1984; Pearson and Knisley 1985; Juliano 1986; Ohba 2008). This may be the case for the carnivorous larvae of *Dytiscus sharpi* (Wehncke), which is also included on the Red Data List of Japan. Therefore, for the purpose of conservation of this endangered species, one of our major tasks should be to understand its foraging ecology. In particular, knowing appropriate dietary items both in the wild and under artificial breeding conditions would be important to preserve the population in the future.

It is well known that the larvae of diving beetles in the genus *Dytiscus* L. are carnivorous. They prey on various animals, such as insect larvae, isopods, amphibian larvae, and fish fry (Blunck 1923*a*; Balduf 1935; Wesenberg–Lund 1943; Jeffries 1988; Johansson and Nilsson 1992). Among these prey animals, amphibian tadpoles are often the major source of food (Balduf 1935; Young 1967; Brodie and Formanowicz 1983). However, in the case of larvae of *Dytiscus harrisii* Kirby, they starved to death even if they were kept with enough tadpoles (Leclair *et al.* 1986). This might be an exceptional case, as they preyed only on case-making caddisfly larvae (Blunck 1916, 1923*a, b*; Leclair *et al.* 1986; Johansson and Nilsson 1992). Thus, evidence so far indicates that food preference is different among *Dytiscus* species, depending on their habitats and available prey species, although the details have not been clarified yet. In addition to food preference, prey size seems to be crucial for the foraging behavior of *Dytiscus* larvae, because it has been reported that young, small larvae preferred smaller tadpoles while larger second and third instars preferred larger prey (Brodie and Formanowicz 1983; Formanowicz 1986). In the present study, we investigated the feeding behavior of *D. sharpi* under laboratory conditions, with particular attention to two factors, prey size and food quantity.

Our main interest is the conservation of endangered species. In contrast to other common *Dytiscus* species, *D. sharpi* is found in restricted narrow areas of Asia, *i.e.*, southern areas in Japan (Wehncke 1875; Sharp 1884) and in part of China (Wu 1937). The restriction of habitats may be one of the major reasons why this species is now highly endangered. For the conservation of *D. sharpi*, it should be an urgent task to understand their ecology. At the same time, another problem is the difficulty in conducting intensive field studies on such rare insects. Therefore, establishing artificial breeding colonies under laboratory conditions will likely be required for these studies. We have already established an aquarium setup for the artificial breeding of *D. sharpi* (Inoda 2003; Inoda and Kamimura 2004); however, there was no quantitative data on the preferred dietary items for the larval growth of *D. sharpi* either in the wild or in captivity. This led us to conduct the present studies.

Dytiscus sharpi mate in late autumn through winter and lay eggs in early spring (Inoda 2003; Inoda *et al.* 2007). Larvae then appear in March and April, and adults emerge in early summer. We conducted field surveys for the candidates for food species in March and April, when the larvae appeared and showed rapid growth. Both the size preference and the number of prey consumed under breeding conditions were then investigated. We ultimately propose a suitable feeding program for the artificial breeding of *D. sharpi*.

Material and Methods

Field Observations. To identify potential prey species of *D. sharpi*, we collected candidate animals from two sites (fallow wetlands) in March and April of 2003, 2005 and 2006 in Japan (Chiba Prefecture). One site was 24 × 12 m (site A), the other was 15 × 10 m (site B), and both were 15–20 cm in water depth. The two sites were under almost the same climatic conditions and were separated by 11 km.

The prey animals were collected using a D–type net 45 cm wide, 40 cm long, 40 cm deep, and with mesh size 0.8 mm. The collection was conducted by a single net sweeping in wetland water and bottom mud every 4 m (total of 10 points in site A) or 3 m (total of 8 points in site B). The species of animals collected were identified and counted according to the following references: aquatic insects (Tsuda 1983; Mori and Kitayama 2002); amphibians (Maeda and Matsui 1999; Uchiyama *et al.* 2002); fishes (Nakabo 2000); and other benthic animals (Ueno 1973). In the case of urodelan larvae, we kept them in an aquarium until metamorphosis in order to identify their species. When we could not identify the species, we used general names.

Some of the collected tadpoles of *Rana ornativentris* (Werner) were used for the following laboratory experiments. Other animals were released back to the collecting sites after the experiments.

Laboratory Experiments. *Diving beetles.* Adult *D. sharpi* used in the experiments were bred and kept in aquaria under outdoor conditions, as described previously (Inoda 2003; Inoda and Kamimura 2004). Under our breeding conditions (20–25°C), we obtained the first instars within 24 hrs after hatching. The beetle larvae were then kept outdoors, according to our previous methods (Inoda and Kamimura 2004), and fed on tadpoles of *R. ornativentris*. In brief, each first instar was kept isolated in a mesh cage (8 × 8 × 8 cm) that was placed in a tank (30 × 20 × 10 cm; water depth 7 cm) in an open aquarium system set outdoors (Inoda and Kamimura 2004). To each first instar reared in a mesh cage for footholds, three to five tadpoles of *R. ornativentris* were supplied daily. Each second or third instar was kept in a tank without mesh cages. Approximately 100 tadpoles of *R. ornativentris* were supplied to each tank every 7–10 days. Tadpoles of body sizes (head–tail length) 10–20 mm, 20–30 mm, and 30–40 mm were provided to the first, second and third instars, respectively. Fresh dechlorinated tap water was supplied automatically every 6 hrs.

We did not control the water temperature or light for the outdoor breeding system. Further details are given in the descriptions of the experiments below. Since third instars (body length approximately 50 mm) prior to pupation require no food, we transferred them to plastic containers (12 × 8 × 8 cm) filled with wet peat moss and held them at room temperature (20–25°C) until emergence. Adult beetles were fed on small pieces of dried sardines or pellets of carp diet thrice weekly. Within 1–2 months after adult eclosion, body size was measured as described below.

Measurements of Body Size of Beetles and Tadpoles. In order to clarify the nutritional efficiency of consuming tadpoles, we measured the body size of adult bred beetles that were kept with only *R. ornativentris* tadpoles for food. To measure the body length, we took photographs of the dorsum of adults with a digital camera (CoolPix 990 or CoolPix 3700, Nikon, Tokyo), and the length of the body, elytra, and pronotum and the width of body were determined from the photographs. The size of *R. ornativentris* tadpoles were measured with calipers or determined from photographs. In order to compare these data with those of wild insects, we used the data of collected beetles reported by Inoda and Kamimura (2004).

Prey Size Preference. Tadpoles of *R. ornativentris* were first classified into three groups according to their size (head-tail length), *i.e.*, S (mean ± SD: 19.3 ± 2.6 mm, n = 196), M (30.5 ± 2.7 mm, n = 196), and L (37.4 ± 2.9 mm, n = 196). Two tadpoles from each group (total of six tadpoles) were then provided to each beetle larva kept in a small tank (12 × 8 × 8 cm, water depth 5 cm) equipped with plastic mesh for footholds. For beetle larvae, 28 first (15.9–22.5 mm), 33 sec (24.0–33.1 mm), and 36 third (34.9–53.0 mm) instars were used. After 12 hrs, we counted the number of remaining tadpoles. We did not use in the final analysis the cases in which all six provided tadpoles were consumed. All the experiments were carried out under laboratory conditions with water temperature maintained at 10–13°C.

Number of Tadpoles Consumed and Instar Development Time. To investigate how many tadpoles *D. sharpi* consumed during each larval stage, we kept each beetle larva isolated individually in a tank (12 × 8 × 8 cm for first or second instars, 13 × 9 × 11 cm for third instars, water depth 5 cm) and fed them with tadpoles of *R. ornativentris*. Ten tadpoles (S-group, 10–20 mm) for first instars, 20 tadpoles (M-group, 20–30 mm) for second instars, or 20 tadpoles (L-group, 30–40 mm) for third instars were provided to each beetle larva (n = 10); the number of tadpoles provided was kept constant during the experiment. Water was exchanged with dechlorinated tap water and any dead prey were removed daily. The experiment was carried out at 18°C under 12L: 12D conditions.

Statistical Analysis. We first analyzed the data with an ANOVA. If there was a statistical significance in F values, the Aspin-Welch t-test was subsequently performed. Otherwise, Student's t-test was used. Data with P values less than 0.05 were considered to be statistically significant on both sides. All statistical calculations were carried out with 'R' software (Ihaka and Gentleman 1996).

Results

Prey Species in the Field. In the beetle habitats of site A, we found 2,625, 5,435 and 6,083 animals in 2003, 2005 and 2006, respectively. Similarly, 5,485, 4,911 and 4,879 animals were found in those respective years at site B. In March and April, we found more than 17 species of prey candidates as well as first instars of *D. sharpi* (Fig. 1). Eleven species were identified and are listed in Table 1. The most abundant species we found was *R. ornativentris* tadpoles (74% and 63% of the total number of animals at sites A and B, respectively). Freshwater isopods (*Asellus hilgendorfi* Bovallius, body length about 10 mm) were also abundant in the field (19% and 32% at sites A and B, respectively). These two species occupied more than 90% of the total number of collected animals.

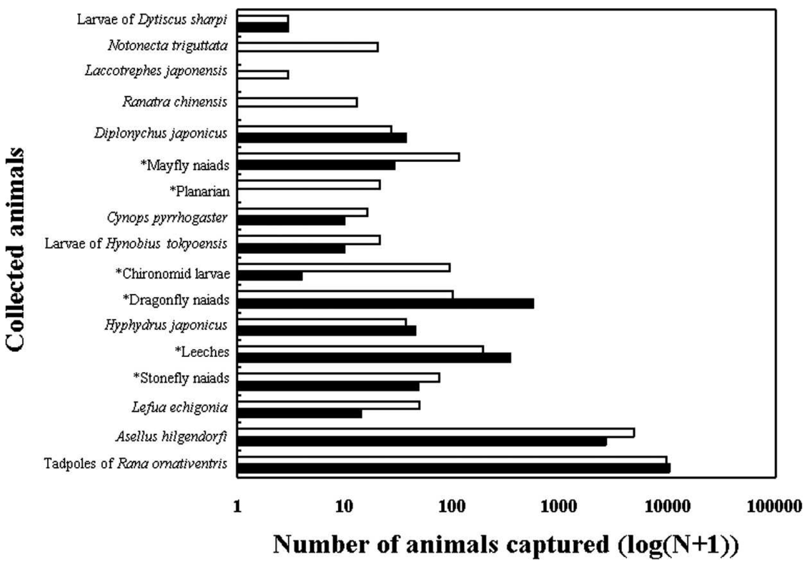

Fig. 1. List of prey candidates found in two wetlands in March and April of 2003, 2005 and 2006. The total number of animals collected at site A (black columns) and site B (white columns) were 2,625 and 5,485 in 2003, 5,435 and 4,911 in 2005 and 6,083 and 4,879 in 2006, respectively. * indicates the prey species that we did not identify.

Nutritional Efficiency of *R. ornativentris* Tadpoles. As shown in Table 2, even if the larvae of *D. sharpi* were supplied with only tadpoles of *R. ornativentris*, we could not find any significant difference in body size compared with wild *D. sharpi* collected in the field (*t*-test).

Size Relationship between Prey and Predator. We analyzed the size correlation between prey (*R. ornativentris* tadpoles) and beetle larvae, as shown in Figure 2. When the larvae were provided with tadpoles of the three size groups (S, M and L), first instars were observed to preferably prey on S-tadpoles with a head–tail length of 19.8 ± 6.0 mm (mean ± SD) ($P < 0.001$). Second instars did not show

Table 1. List of the identified species found in two wetlands in Chiba Prefecture, Japan in March and April of 2003, 2005 and 2006.

Order	Family	Species
Isopoda	Asellidae	*Asellus hilgendorfi* Bovallius
Hemiptera	Belostomatidae	*Diplonychus japonicus* Vuillefroy
	Nepidae	*Laccotrephes japonensis* Scott
	Nepidae	*Ranatra chinensis* Mayer
	Notonectidae	*Notonecta triguttata* Motschulsky
Coleoptera	Dytiscidae	*Dytiscus sharpi* Wehncke
	Dytiscidae	*Hyphydrus japonicus* Sharp
Caudata	Salamandridae	*Cynops pyrrhogaster* Boie
	Salamandridae	*Hynobius tokyoensis* Tago
Anura	Ranidae	*Rana ornativentris* Werner
Cypriniformes	Cobitidae	*Lefua echigonia* Jordan and Richardson

Table 2. Comparison of body size between reared and wild *D. sharpi* adults. Data for wild adults were taken from Inoda and Kamimura (2004). Number in parentheses indicates sample size. Values are mean ± SD.

		Body length [mm]	Elytral length [mm]	Pronotal length [mm]	Body width [mm]
Female	Bred (45)	30.4 ± 1.6	22.7 ± 1.4	4.4 ± 0.7	15.2 ± 0.9
	*Wild (14)	30.2 ± 1.9	22.3 ± 0.9	4.6 ± 0.6	15.1 ± 0.5
Male	Bred (45)	31.2 ± 2.1	24.0 ± 1.4	5.2 ± 0.6	15.9 ± 1.1
	*Wild (13)	31.1 ± 1.7	23.6 ± 1.2	5.2 ± 0.4	15.9 ± 0.8

any specific size preference. In contrast, third instars preyed on L-tadpoles with 30.9 ± 6.4 mm head-tail length ($P < 0.05$). Thus, our observations indicate that there is some size-dependency of preferred prey correlated with the larval stages of *D. sharpi*.

Number of Tadpoles Consumed and Instar Development Time. As shown in Figure 3 and Table 3, first instars of *D. sharpi* typically fed on 5 tadpoles per day.

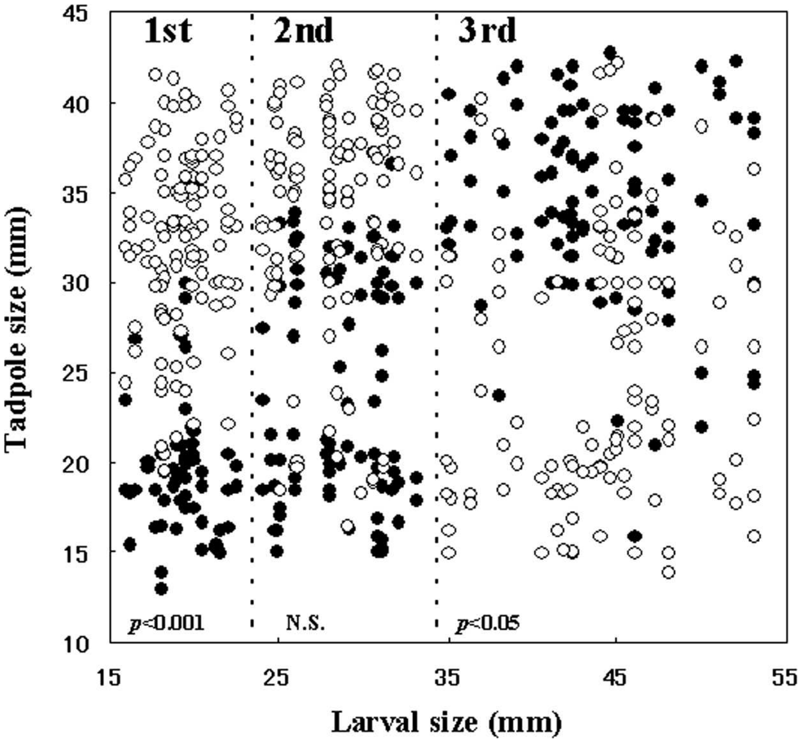

Fig. 2. Relationship of *D. sharpi* larval size and prey tadpole size. Black and white circles indicate eaten and surviving tadpoles in a 12 hr period, respectively. There was a significant difference between the number of eaten and surviving tadpoles in the case of first ($P < 0.001$) and third ($P < 0.05$) instars. No significant difference was observed in the case of second instars.

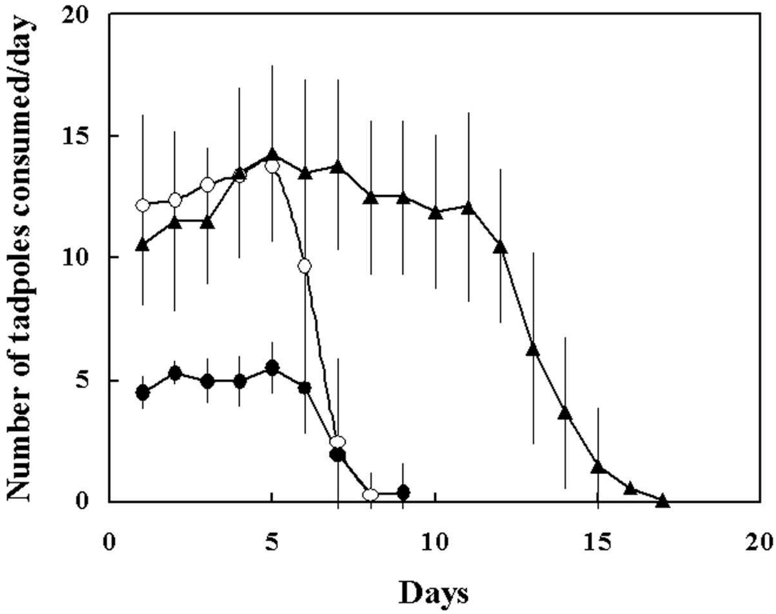

Fig. 3. Total number of tadpoles consumed by first (black circles), second (white circles) and third (triangles) instars of *D. sharpi*. Values indicate the mean ± SD of 10 larvae.

In total, first instars preyed on 33 ± 5 (mean ± SD) tadpoles and took about 7 d to develop. Second and third instars preyed on 77 ± 6 and 160 ± 24 tadpoles, respectively. The development periods of the second and third instars were on average 7 and 14 d, respectively.

Discussion

Formanowicz (1987) suggested that the larvae of *Dytiscus verticalis* Say used mechanical stimuli or some chemicals from their prey instead of visual cues to find prey. He showed that beetle larvae oriented towards and approached moving prey, but such signals were not enough to evoke attacking behavior. Thus, some chemical cues are likely required. In the case of *D. sharpi*, however, the motion of prey (mechanical signals) seemed to be enough to start the foraging behavior, based on our preliminary observations under laboratory conditions. The beetle

Table 3. Number of tadpoles consumed and instar development period of *D. sharpi* at 18°C, 12L : 12D photoperiod. Values are the mean ± SD of 10 insects. Numbers in parentheses indicate range (minimum–maximum).

	Larval stages			
	1st	2nd	3rd	1st-3rd
Number of tadpoles consumed	32.7 ± 4.7 (26–41)	77.3 ± 6.1 (67–86)	160.4 ± 23.5 (132–200)	270.4 ± 29.6 (232–316)
Larval period [Days]	6.5 ± 1.1 (5–9)	6.6 ± 0.7 (6–8)	14.4 ± 1.4 (12–17)	27.5 ± 1.8 (25–30)

larvae attacked and preyed on various kinds of animals, regardless of whether the prey animals actually exist in the wild habitats, *e.g.*, amphibian larvae, amphibian adults, arthropods, and fishes. Therefore, the larvae of diving beetles would be typical non–specific feeders. This implies that any small moving animals that are abundant in the field from March to April, when *D. sharpi* larvae appear, would be major prey. In our field observations, we often found that the larvae preyed mainly on the tadpoles but also attacked any other potential prey that moved. Therefore, we assumed that the abundant *R. ornativentris* tadpoles would serve as the main prey for the larvae of *D. sharpi*, as has been shown for other *Dytiscus* species (Balduf 1935; Young 1967; Brodie and Formanowicz 1983). Since sufficient numbers of tadpoles of *R. ornativentris* are usually available in the *Dytiscus* habitats and we can keep them feeding on *R. ornativentris* in a simple aquarium system, *R. ornativentris* tadpoles seem to be one of the most appropriate dietary items for *D. sharpi* larvae. In general, we can expect that any predator would adapt their preference for prey to those that promise optimal efficiency for capturing, feeding, digesting, and nutrition (Dethier 1976).

We found a positive correlation between the size of *D. sharpi* larvae and prey size, *i.e.*, larger larvae prefer larger prey than smaller ones (Fig. 2), as has been shown for other *Dytiscus* species (Brodie and Formanowicz 1983). Although the detailed mechanism on how the larvae choose prey size is not clear yet, such preference may be partially related to likelihood of the beetle larva encountering the prey (Formanowicz 1982, 1984). Being accidentally injured while hunting larger prey would be a probable factor to be considered (Brodie and Formanowicz 1983); however, how this is programmed into the predatory behavior of the insect should be investigated. In addition, we propose here another possible factor, namely the efficiency of hunting. Beetle larvae catch prey using a pair of mandibles, and they feed by injecting digestive enzymes through their mandibles into the prey (Formanowicz and Brodie 1981, 1982). Feeding is done by sucking up enzymatically–digested contents. This indicates that beetle mandibles have two important functions for feeding and hunting. Furthermore, it would imply that beetles need to find prey within a certain range of body size suitable for the width of their mandibles for efficient hunting. Thus, the prey size preference would be tightly correlated with such size restrictions, *i.e.*, whether or not they can catch it with their mandibles.

Blunck (1923*a*) reported that *Dytiscus* larvae required a relatively high amount of food during their development. Larvae of *Dytiscus marginalis* L. preying on tadpoles consumed up to 900 (Wesenberg-Lund 1943) and 300 (Blunck 1923*a*). In the present study, we also found that the larvae of *D. sharpi* consumed 300 or more *R. ornativentris* tadpoles during their development.

Each female *R. ornativentris* spawns a mass of 1,000–1,900 eggs (Maeda and Matsui 1999). Under laboratory breeding conditions, we observed that females of *D. sharpi* lay approximately 50–100 eggs (Inoda 2003; Inoda and Kamimura 2004). Combined with the present results of the total consumption of tadpoles required for the full development of *D. sharpi* larvae, we calculate that we must keep all the tadpoles from eggs laid by about 19 female frogs in order to maintain all the hatched larvae from one *D. sharpi* female.

We developed an artificial rearing system for *D. sharpi* larvae in a previous study (Inoda and Kamimura 2004); however, in that study, food requirements were not investigated in detail. From the present quantitative analysis of dietary efficiency, we propose a new effective feeding program for the endangered *D. sharpi*, as follows.

i) Before starting to rear the beetle larvae, approximately 300 *R. ornativentris* tadpoles per *D. sharpi* larva must be collected.
ii) After obtaining the first instar, more than five *R. ornativentris* tadpoles with a head–tail length of 10–20 mm are to be provided to each beetle larva daily.
iii) For the second and third instars, more than 10–15 *R. ornativentris* tadpoles with a head–tail length of 20–30 mm or 30–40 mm, respectively, are to be provided to each beetle larva daily.

Using this feeding program with a water temperature of 18°C, which is almost the same as that in their habitat during the development season (Inoda *et al.* 2007), 7, 7 and 14 d are expected periods for the development of first, second and third instars, respectively. An open aquarium system (Inoda and Kamimura 2004) combined with our feeding program should be one of the most effective conditions to maintain this endangered diving beetle. Such a breeding system is expected to be useful for further basic studies and for the conservation of water beetles in the future.

Though we demonstrated that *R. ornativentris* tadpoles are an important food for the larvae of *D. sharpi*, the population of *R. ornativentris* has been decreasing rapidly due to environmental problems, and it also is listed in the Red Data Book (Category: Rank C) of the Chiba Prefecture of Japan (Chiba Prefecture 2000). Our present and previous studies (Inoda 2003; Inoda *et al.* 2007) indicate that even a slight failure to synchronize the timing of larval hatching and development with the growth of prey tadpoles would induce crucial and irreversible damage to the population of *D. sharpi*. This indicates that, for the conservation of *D. sharpi*, we must conserve the environment for *R. ornativentris* as well. Searching for other possible prey candidates instead of *R. ornativentris* tadpoles in the field and testing them under laboratory breeding conditions as described here would be indispensable. Since we mainly conducted laboratory experiments, the comparison of feeding behavior between reared and wild insects should be required to know their detailed bionomics.

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