

Research Article

Feeding Preferences of the Endangered Diving Beetle *Cybister tripunctatus orientalis* Gschwendtner (Coleoptera: Dytiscidae)

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Received 14 September 2011; Revised 24 November 2011; Accepted 7 December 2011

Academic Editor: Martin H. Villet

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The numbers of *Cybister tripunctatus orientalis* Gschwendtner diving beetles are declining in most regions of Japan, and it is included in the Red Data List of species in 34 of 47 prefectures of Japan. However, basic ecological information about *C. tripunctatus orientalis*, such as its feeding habits, remains unknown. In order to elucidate the feeding habits of *C. tripunctatus orientalis* larvae, feeding preference experiments were carried out in 2nd and 3rd instar larvae. The number of Odonata nymphs consumed was significantly higher than the number of tadpoles consumed, indicating that *C. tripunctatus orientalis* larvae prefer Odonata nymphs to tadpoles. In addition, all the first instar larvae of *C. tripunctatus orientalis* developed into second instars when they were supplied with motionless Odonata nymphs, but their survival rate was lower when they were supplied with motionless tadpoles. These results suggest that *C. tripunctatus orientalis* larvae prefer insects to vertebrates.

1. Introduction

Cybister tripunctatus orientalis Gschwendtner (adult body length: 24–29 mm) is found in China, the Korean Peninsula, Taiwan, and Japan excluding Hokkaido [1]. The numbers of *C. tripunctatus orientalis* are declining in most regions of Japan, and it is included in the Red Data List of species in 34 of 47 prefectures of Japan [2, 3]. *Cybister tripunctatus orientalis* has become extinct in Tokyo, Kanagawa, Aichi, Kyoto, Osaka, Wakayama, and Hyogo (it was rediscovered in Hyogo in 2010: [4]). Contributing factors, such as a decreasing number of suitable aquatic habitats due to the abandonment of rice paddies, water pollution, pesticide application, and invasion by alien species, are of great concern [2, 5]. In addition, the sizes of predatory invertebrate populations are limited by their food resources, as is true for any predatory insect [6–8]. Thus, understanding their trophic ecology is necessary to support an insect conservation program. However, basic ecological information about *C. tripunctatus orientalis*, such as their feeding habits, remains unknown.

A number of descriptive reports [9–12] have suggested that *Cybister* larvae feed on tadpoles, fish, and aquatic

insects. In studies of *C. tripunctatus orientalis*, Kunimoto [13] saw 3rd instar larvae capturing tadpoles and Odonata larvae (*Pantala flavescens*) in rice fields in Tottori, western Japan. Ohba [14, 15] revealed the larval feeding habits of two congeneric *Cybister* species based on a field census and rearing experiment: *C. brevis* Aubé and *C. chinensis* Motschulsky (formerly *Cybister japonicus* Sharp, see Nilsson and Petrov [16]) larvae preyed mainly on aquatic insects and did not eat vertebrate animals such as tadpoles, except for the 3rd instar of *C. chinensis*. Moreover, these studies showed that the results of feeding preference experiments performed under laboratory conditions were in accordance with field observations of the *Cybister* species and their growth performance [14, 15]. Therefore, feeding preference experiments performed under laboratory conditions can contribute to deducing the natural feeding habits of *Cybister* species. The objective of this study was to reveal whether the larvae of *C. tripunctatus orientalis* prefer invertebrate prey (Odonata nymph) over vertebrate prey (tadpole). For that purpose, two laboratory experiments were carried out in order to determine the feeding preferences of *C. tripunctatus orientalis* larvae.

2. Materials and Methods

2.1. Study Animals. Three male and two female *C. tripunctatus orientalis* adults were collected as breeding stock from an irrigation pond in southern Kochi, Shikoku, Japan, in September 2010 and kept in an aquarium (45 cm × 34 cm in dimension, 20 cm in height) maintained under natural water temperature and day length conditions for overwintering. From April 2011 onwards, they were maintained at a water temperature of 25°C under a 16L : 8D light cycle to stimulate reproduction. River gravel was laid onto the bottom of the aquarium in a 2 cm thick layer, and dechlorinated tap water was added to a depth of 15 cm over the gravel surface. Three water hyacinths (*Eichhornia crassipes*; ca. 5 cm in stock diameter) were planted in the aquarium as oviposition sites. Hatched larvae were reared individually in small plastic containers (10 cm diameter × 10 cm in height) filled to a depth of 2 cm with dechlorinated tap water and kept under natural temperature and day length conditions from June to July 2011. Larvae of *Culex* spp., chironomids, and notonectid nymphs were supplied to the *C. tripunctatus orientalis* larvae as food for the first experiment. The prey animals used in this study were collected from rice fields and irrigation ponds.

2.2. First Experiment. To investigate the feeding preferences of *C. tripunctatus orientalis*, a feeding preference experiment was conducted (see Ohba [14, 15]) using 2nd and 3rd instar larvae. As the first instar larvae did not capture live tadpoles in the preliminary experiment, we did not use first instar larvae in the feeding preference experiment. Small damselfly nymphs (Platynemididae: *Copera* spp. or Lestidae: *Lestes* spp., 15–20 mm) and large dragonfly nymphs (*Sympetrum* spp. 20–30 mm) were provided as food to the 2nd and 3rd instar larvae, respectively. In addition, small (snout to vent length: 10–20 mm) and large (20–30 mm) tadpoles of the tree frog *Hyla japonica* were provided as food to these larval instars, respectively. The prey density in each plastic container was kept constant (3 tadpoles and 3 Odonata nymphs). Each beetle larva was fasted for a day before the experiment. Before the start of the experiment, the Odonata nymphs were fed larval *Aedes* spp. on a daily *ad libitum* basis in order to prevent intra- and interspecific predation (attacking the tadpoles) during the experiment. The number of carcasses (consumed by the *C. tripunctatus orientalis* larva) of each prey animal was counted at 24 hours after the beginning of the experiment. The experiments using the 2nd and 3rd instar nymphs were replicated 10 and 14 times, respectively. To determine the diet choices of *C. tripunctatus orientalis* larvae, the Wilcoxon signed-rank test was used to compare the number of prey consumed between the tadpoles and Odonata nymphs for each larval instar. In this case, paired nonparametric test should be applied to this data because of discrete-valued data.

2.3. Second Experiment. In the preliminary experiment, *C. tripunctatus orientalis* larvae hardly ever consumed tadpoles but they did routinely eat Odonata nymphs. This might have been caused by different escape capabilities of tadpoles and Odonata nymphs. To examine only the influence each

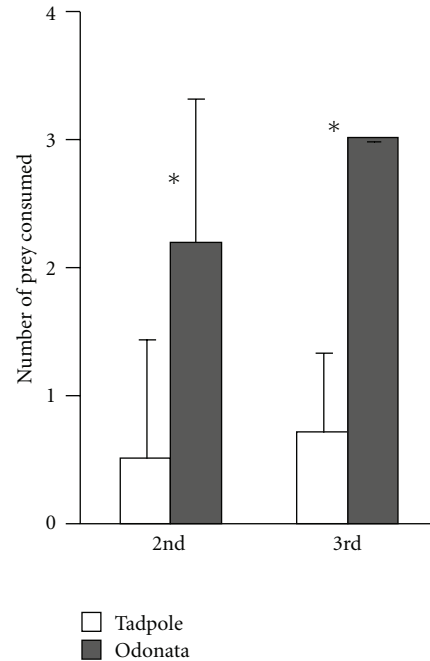


FIGURE 1: The number of tadpoles and Odonata nymphs consumed by *Cybister tripunctatus orientalis* larvae. * $P < 0.05$, the Wilcoxon signed-rank test. Data are shown as the mean + S.D.

prey item on the development of *C. tripunctatus orientalis* larvae when the prey were prevented from escaping, motionless tadpoles and motionless Odonata nymphs were used. The bases of the tadpole tails (*H. japonica*, ca. 10 mm) or the thoraces of the Odonata nymphs (*Lestes* spp. ca. 15 mm) were squeezed using forceps for 5 seconds to immobilize them. A first instar *C. tripunctatus orientalis* larva and one motionless prey specimen were put into a small plastic container. The individual prey were exchanged for new prey every day, and this process was continued for 15 days or until the *C. tripunctatus orientalis* larva died. The motionless tadpole and motionless Odonata nymph treatments were replicated 6 times each. Survival analysis was used to test for survival curve differences between the motionless tadpoles and motionless Odonata nymphs. The Kaplan-Meier method for estimating survival and the nonparametric Mantel-Cox log-rank test were used. Survival analysis has been regularly employed in medical science to analyze “incomplete” data recorded before the termination of the event of interest. Because many insects died before the present experiments were terminated, the incomplete data thus resulting is not suitable for traditional nonparametric techniques (e.g., Moore and Townsend [17]). A statistically different significance was assumed to be at $P < 0.05$. All statistical tests were conducted using the Statcel [18].

3. Results and Discussion

In the first experiment, the number of Odonata nymphs consumed was significantly higher than the number of tadpoles consumed in 2nd and 3rd instars (the Wilcoxon signed-rank test, 2nd: $Z = 2.55$; 3rd: $Z = 2.98$, $P < 0.02$ for both; Figure 1).

Although we did not carry out any field observations, it is assumed that *C. tripunctatus orientalis* larvae consume mostly aquatic invertebrates in their natural habitats. However, Kunimoto [13] recorded 3rd instar larvae of *C. tripunctatus orientalis* capturing tadpoles in rice fields. This discrepancy would most likely have been caused by differences in prey density; that is, their study was carried out in June, when there is a high density of tadpoles in rice fields [13]. According to Ohba [15], the emergence of first and second instar larvae of closely related species, *Cybister chinensis* Motschulsky, coincided with the appearance period of Odonata nymphs and tadpoles but these larvae fed on Odonata nymphs and did not eat tadpoles. Therefore, larvae of *C. tripunctatus orientalis* may not eat tadpoles well in their fields. In fact, *C. tripunctatus orientalis* larvae consumed more Odonata nymphs than tadpoles when supplied with the same number of Odonata nymphs and tadpoles (Figure 1). Thus, the 2nd and 3rd instar larvae of *C. tripunctatus orientalis* preferred Odonata nymphs over tadpoles, as is also observed for closely related species, *Cybister brevis* Aubé, larvae [14].

In the second experiment, the survival rates of *C. tripunctatus orientalis* larvae differed significantly between those feeding on motionless tadpole and Odonata nymph treatments (survival analysis, Mantel-Cox $\chi^2 = 5.87$, $P = 0.015$). In larvae fed motionless tadpoles, four died after 12.3 ± 4.27 (mean \pm S.D.) days and two developed into 2nd instar larvae after 14.5 ± 0.71 days. On the contrary, all six larvae fed motionless Odonata nymphs developed into 2nd instar larvae after 6.2 ± 0.41 days. The mean larval duration of the first instar was longer in the motionless tadpole treatment than in the motionless Odonata nymph treatment irrespective of survival. The results show that *C. tripunctatus orientalis* larvae can consume tadpoles but obtain insufficient levels of nutrients from them for optimal development. Interestingly, *C. brevis* larvae died from starvation after being supplied with only motionless tadpoles, indicating that *C. brevis* larvae do not consume tadpoles; not because they cannot capture tadpoles but because they dislike and/or do not recognize tadpoles as prey animals [14]. These different feeding habits might be attributable to differences in digestive enzymes between the two species. This should be examined in future studies. These results strongly suggest that environments with abundant aquatic invertebrates are favorable for maintaining *C. tripunctatus orientalis* populations.

Rice fields are an important breeding habitat for aquatic insects [19] including *C. tripunctatus orientalis* in Japan. However, the species diversity in rice fields has been declining due to recent land consolidation, the modification of traditional earth ditches to U-shaped concrete ditches in Japan [5, 20]. Therefore, poorly drained paddies, which are wet in winter and kept flooded throughout summer, are suitable to conserve the aquatic invertebrates as well as *C. tripunctatus orientalis*.

Acknowledgments

The authors thank N. Katayama and Y. Sakai for their help with the beetle keeping and N. Sonoda and Y. Ohba for their kind assistance during this study.

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