Feeding habits of the diving beetle larvae, *Cybister brevis* Aubé (Coleoptera: Dytiscidae) in Japanese wetlands

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(Received 4 February 2009; Accepted 1 April 2009)

Abstract

A number of descriptive reports suggest that *Cybister* larvae feed on tadpoles, fish, and aquatic insects; however, no quantitative study on their feeding habits has been reported. In order to elucidate the feeding ecology of *C. brevis* larvae, field observations and laboratory experiments were carried out. In the field, all *C. brevis* larvae fed on invertebrates, such as insects and isopods, but did not eat vertebrates, such as fish and anuran larvae. A rearing experiment demonstrated that all *C. brevis* larvae provided with tadpoles died. Larvae provided with Odonata nymphs had a longer total body length than larvae reared with a mixture of tadpoles and Odonata nymphs. In addition, larvae of *C. brevis* could search for and eat motionless Odonata nymphs, but all larvae died from starvation when they were supplied with motionless tadpoles. These results suggested that *C. brevis* larvae mainly preyed upon invertebrate animals and did not eat vertebrate animals, such as tadpoles and fish.

Key words: Feeding habit; diving beetle larva; *Cybister brevis*; *Culex*

INTRODUCTION

In wetlands, diving beetles (Dytiscidae) are important predators and are often at the upper end of the food web in aquatic communities. The larvae of some species are regarded as effective predators of mosquito larvae (Bay, 1974; Berman et al., 2000; Lundkvist et al., 2003). The genus *Cybister*, one of the Dytiscidae, is a relatively large species. Seven *Cybister* species are distributed across the Japanese archipelago.

*Cybister brevis* Aubé (20–25 mm in body length) is distributed in China, the Korean Peninsula, and Japan, excluding Hokkaido and the Ryukyu Islands (Mori and Kitayama, 2002). They live in rice paddy water systems and reproduce from May to August in both rice fields and ponds (Saijo, 2001, 2002). The numbers of *C. brevis* are declining in some regions of Japan, and this species is designated in the Red Data List of species in 18 of 47 prefectures (Association of Wildlife Research & EnVision, 2007). Contributing factors, such as decreasing amounts of suitable aquatic habitats, abandonment of rice paddies, water pollution, pesticide application, and invasion by alien species are of great concern (e.g., Nishihara et al., 2006). Moreover, the population size of predatory invertebrates is limited by its food resources, as for many other predatory insects (e.g., Lenki, 1984; Pearson and Knisley, 1985; Juliano, 1986). Thus, understanding the feeding habits of *C. brevis* in their natural environment is significant for conservation efforts.

A number of descriptive reports (e.g., Ichikawa, 1984, 2007; Tsuzuki et al., 1999; Uchiyama, 2005) suggest that *Cybister* larva feed on tadpoles, fish, and aquatic insects; however, no quantitative studies on the feeding habits of *C. brevis* have been performed. In the present study, in order to examine the feeding ecology of *C. brevis*, field observations and laboratory experiments were carried out.

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DOI: 10.1303/aez.2009.447
MATERIALS AND METHODS

Prey composition of *C. brevis* larvae in the field. To investigate the dietary habits of *C. brevis*, field censuses were conducted at intervals of 1 to 2 days in rice paddy water systems in eastern Shimane, Japan, from 3 May to 28 July 2008. Censuses were conducted along the ridges around rice paddy water systems (about 100 m²) and irrigation ponds (about 20 m²). In the daytime, observation of the beetles was difficult because of the reflection of sunlight on the water surface. Beetle individuals were observed directly in the field using a flashlight (11,000 lx) from 20:00 to 02:00. The flashlight did not interfere with the foraging behaviors of beetle larvae as they did not stop feeding or hunting prey (S. Ohba, unpublished data). In contrast, it was impossible to observe the dietary components of beetle adults because they disappeared into the water as soon as the investigator approached. When beetle larvae were found, it was noted whether they were holding prey in their mandibles (Fig. 1). The body widths of the prey were measured as indices of prey body size, and the head widths of *C. brevis* larvae were measured with calipers. After measurement, the *C. brevis* larvae were released immediately at their point of capture. *C. brevis* larvae were assigned to instars based on the head width data obtained from preliminary survey (S. Ohba, unpublished data) as follows: first instar, 1.4–1.6 mm; second instar, 2.3–2.7 mm; and third instar, 2.9–4.3 mm. Prey that had deteriorated by digestion were excluded from the measurements. The type of prey held in the mandibles was recorded as a dietary component and preserved in 70% ethyl alcohol for later identification. Spearman’s rank correlation coefficient was used to evaluate the association between head widths of *C. brevis* larva and the body widths of their prey (*n* = 58).

Rearing experiment. Five male and five female *C. brevis* adults were collected as breeding stock from an irrigation pond in eastern Shimane, Japan, in April 2008, and kept in an aquarium (45 cm × 34 cm, 20 cm height) maintained at 27.4 ± 0.11°C (SE) water temperature and a 16L8D light cycle. River gravel was laid on the bottom of the aquarium in a 20 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) and six narrow leaf Amazon swords *Echinodorus amazonicus* (ca. 10 cm in plant length) were planted in the aquarium as oviposition sites.

Hatched larvae were reared individually in small plastic containers (6 cm × 6 cm sides, 5 cm height) with 1 hole (5 mm diameter) in the bottom, and the tops were covered with a plastic board (Fig. 2a). The bottom hole in each plastic container was covered with net (3 mm mesh) and filled with a 1 cm thick layer of river gravel. To prevent the water quality from deteriorating and to maintain the same water quality in each small plastic container, all containers were placed in large aquarium (63.5 cm × 43.9 cm × 22.6 cm) kept at 29.3 ± 0.07°C water temperature and a 16L8D light cycle. The aquarium was filled with water to a depth of 15 cm, and aeration, one tube with one filtering device-airstone per air pump, was implemented (see Ohba, 2008). All plastic containers were fixed within the aquarium to keep the water depth at 3 cm (Fig. 2b).

Experiments were conducted separately for three prey treatments: tadpole, Odonata nymph, and a tadpole-Odonata nymph mixture. The tadpole, Odonata nymph, and tadpole-Odonata nymph mixture treatments were replicated 5, 16, and 12 times, respectively. Because it is known that some insect predators provided with mixture prey animals had higher performance than those supplied with a single type of prey (e.g., Sonoda et al., 1992; Zanuncio et al., 2001), the tadpole-Odonata nymph mixture treatment was conducted. Prey animals in this...
experiment were collected from rice fields and irrigation ponds. Small damselfly nymphs (Platycnemididae: Coptera spp. and Lestidae: Lestes spp., <15 mm), medium damselfly nymphs (same species, 15–20 mm), and large dragonfly nymphs (Libellulidae: Orthetrum albistylum speciosum, Sympetrum frequens, S. infuscatum; Aeshnidae: Planaeschnu milnei, and Anax parthenope, 20–30 mm) were provided as food to 1st, 2nd, and 3rd instars, respectively. Small (<7 mm in snout to vent length), medium (10–20 mm), and large (20–30 mm) tadpoles of the pond frog R. nigromaculata were provided as food to 1st, 2nd, and 3rd instars, respectively. In the tadpole-Odonata nymph mixture treatment, the same number of tadpoles and Odonata individuals were provided. The density of prey in each plastic container was kept constant (6 tadpoles for tadpole treatment, 6 Odonata nymphs for Odonata nymph treatment, and 3 tadpoles and 3 Odonata nymphs for tadpole-Odonata nymph mixture treatment). Prey density levels were set to supply enough food for C. brevis larvae during their development. To maintain a constant prey density in each larval stage, the number of prey was checked each day and additional prey were provided as necessary. Simultaneously, dead prey were removed immediately from containers. Third instar larva of all dytiscid beetles do not eat any prey just before pupation (Tsuzuki et al., 1999). Thus, 3rd instar larva that did not eat prey within one hour after it was provided were moved to a cup (10 cm diameter×10 cm height) filled with peat moss in order to confirm their pupation. The day when 3rd instar burrowed into the peat moss was recorded as the last day of the larval period. New adults emerging from the peat moss were examined to determine their sex, and their total body length was measured using calipers. After the above-mentioned field censuses were finished, all beetle adults were released in the irrigation pond from which beetles had been captured.

To compare the survival rates of first instar larvae of C. brevis, the χ² test with sequential Bonferroni test was used among three prey treatments. Tadpole treatment was excluded from analysis of the larval period and adult body size because all 1st instar larvae died (see Results). To evaluate the effect of prey type on the adult total body length, two-way analysis of variance (ANOVA) with prey (Odonata nymphs and tadpole-Odonata nymph mixture) and sex was performed. The larval period of C. brevis in the two prey treatments was compared using repeated-measures two-way ANOVA, with prey (Odonata nymphs and tadpole-Odonata nymph mixture) and sex as the between-subject factors and larval stage (1st–3rd instar) as the within-subject factor. Because Mauchly’s test did not indicate a significant violation of the assumption of sphericity in the analysis of the larval period (p=0.07), significance levels for within-subject effects were not corrected using the Greenhouse-Geisser method for the degrees of freedom.
To determine the diet choice of *C. brevis* larvae, the number of each prey item consumed in tadpole-Odonata nymph mixture treatment was recorded for each larval instar. The paired *t*-test was used to compare the number of prey consumed between tadpoles and Odonata nymphs for each larval instar. Log₁₀ transformations for exact values were made in order to standardize and normalize the variances, if necessary, to satisfy the assumptions of the ANOVA model.

**Motionless prey.** In the rearing experiment and field census, *C. brevis* larvae hardly ever consumed tadpoles but they routinely ate Odonata nymphs. This may have been caused by differences in escaping ability between tadpoles and Odonata nymphs. To examine the effect of each prey item on the development of *C. brevis* larvae when the prey was disabled from escaping, motionless tadpoles and motionless Odonata nymphs were used. The base part of tadpole tails (*R. nigromaculata*, ca. 10 mm) or the thorax of Odonata nymphs (*Lestes* spp. ca. 15 mm) was squeezed using forceps for 5 seconds to immobilize them. A 1st instar larva of *C. brevis* and one motionless prey were put into a small, plastic container (Fig. 2a). Individual prey were exchanged for new prey every day, and this process was continued until the beetle larvae became 2nd instar or died. Survival analysis was used to test for survival curve differences between motionless tadpoles and motionless Odonata nymphs. The Kaplan-Meier method of estimating survival function and the nonparametric Mantel-Cox log-rank test were used. In this analysis, transforming to the 2nd instar was regarded as censoring. Statistical significance was set at 0.05. All statistical tests were conducted using JMP software (JMP version 7.0, SAS Institute, 2007).

**RESULTS**

**Prey composition of *C. brevis* larvae in the field**

A significant positive correlation was found between the head width of *C. brevis* larvae and their prey (*rs* = 0.489, *p* < 0.001, Fig. 3). All *C. brevis* larvae fed on insects and isopoda but did not utilize vertebrates, such as fish and anuran larvae (Table 1). The 1st and 2nd instar fed primarily on Ephemeroptera, and Ephemeroptera and Coleoptera, respectively, including cannibalism. First instar larvae also fed on the larvae of *Culex mimeticus* (Diptera). On the other hand, 3rd instar fed frequently on Heteroptera, including mainly backswimmer (*Notonecta triguttata*) and Odonata nymphs.

![Fig. 3. Correlation relationship between the head width of *C. brevis* larvae and their prey (Spearman's rank correlation coefficient).](image)

![Table 1. Types and percentages of dietary components consumed by *C. brevis* larvae in the field](table)

<table>
<thead>
<tr>
<th>1st instar</th>
<th>2nd instar</th>
<th>3rd instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=10)</td>
<td>(n=11)</td>
<td>(n=46)</td>
</tr>
</tbody>
</table>

**Insecta**

- **Ephemeroptera**
  - Baetidae nymph: 30.0
  - Odonata: —
    - Aeshnidae nymph: —
    - Libellulidae nymph: —
    - Damsel fly nymph: —
  - Orthoptera: —
    - Gryllotalpa orientalis: —
  - Heteroptera: Notonecta triguttata: 20.0
    - Appasus major: —
  - Coleoptera: Cybister brevis larvae: —
    - *Hyphorus japonicus* larva: 10.0
    - Hydrophilidae larva: —
    - Unknown: —
  - Diptera: Culex mimeticus larva: 10.0
    - Chironomidae larva: —
  - Trichoptera: *Molanna moesta* larva: —
    - Unknown: 10.0
  - Isopoda: *Asellus hilgendorfi*: 20.0

| Total | 100 | 100 | 100 |
Rearing experiment

From the rearing experiment, the survival rate of larvae up to emergence of the 2nd instar in the tadpole treatment (0%, n=5) was significantly lower than those in the Odonata nymph treatment (100%, n=16) and tadpole-Odonata nymph mixture treatment (93%, n=14) (χ² test by sequential Bonferroni tests, χ² > 14.7, d.f. = 2, p < 0.05, for both). The effects of two prey diets, Odonata nymph and the tadpole-Odonata nymph mixture, on the development of *C. brevis* larvae, were evaluated using adult total body length. Two-way ANOVA revealed that prey and sex effects were significant but prey-by-sex interaction was not significant (prey: F₁, 22 = 9.89, p = 0.005; sex: F₁, 22 = 6.48, p = 0.02; prey × sex: F₁, 22 = 0.28, p = 0.60). In both sexes, individuals in the Odonata nymph treatment had longer total body length than those in the tadpole-Odonata nymph mixture (Fig. 4a).

For the larval period, repeated-measures two-way ANOVA revealed that sex and larval stage effects were significant but larval stage-by-prey interaction, and larval stage-by-sex interaction effects were not significant (prey: F₁, 24 = 2.34, p = 0.14; sex: F₁, 24 = 5.07, p = 0.034; larval stage: F₂, 48 = 3.97, p = 0.03; larval stage × prey: F₂, 48 = 0.05, p = 0.95; larval stage × sex: F₂, 48 = 1.39, p = 0.26 for log-transformed data). Differences between the two prey treatments were not found between sexes (Table 2). In the tadpole-Odonata nymph mixture treatment, the number of Odonata nymphs consumed was significantly more than the number of tadpoles consumed throughout all instars (paired t-test, 1st: t₁,₁₁ = 11.00; 2nd: t₁,₁₁ = 13.35; 3rd: t₁,₁₁ = 5.23, p < 0.0001 for all; Fig. 4).

Motionless prey

The survival rates of *C. brevis* larvae differed between the motionless tadpole and Odonata nymph treatment (Survival analysis, Mantel-Cox χ² = 5.41, p = 0.02). In motionless tadpole treatment, four larvae did not bite the tadpole, and all larvae died from starvation after 3.25 ± 0.50 (mean ± SD) days. In contrast, four larvae in the motionless Odonata nymph treatment bit Odonata nymphs after detecting them. All individuals became 2nd instar after 5.50 ± 0.58 days.

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**Table 2. Larval period in each instar**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>Larval instar (average days ± SD (range))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st</td>
</tr>
<tr>
<td>Odonata nymph</td>
<td>Male</td>
<td>4.71 ± 0.29 (3–5)</td>
</tr>
<tr>
<td>Tadpole-Odonata nymph mixture</td>
<td>5</td>
<td>5.00 ± 0.45 (4–6)</td>
</tr>
<tr>
<td>Odonata nymph</td>
<td>Female</td>
<td>6.14 ± 0.51 (5–9)</td>
</tr>
<tr>
<td>Tadpole-Odonata nymph mixture</td>
<td>8</td>
<td>5.25 ± 0.25 (4–6)</td>
</tr>
</tbody>
</table>

*a Tadpole treatment was excluded because all 1st instar larvae died (n=5).
DISCUSSION

The present study examined the feeding habits of larvae of *Cybister brevis*. The body size of prey animals increased as the larvae of *C. brevis* grew, as for many other predatory insects (Cloarec, 1992; Perez Goodwyn, 2001; Ohba et al., 2008). All *C. brevis* larvae fed on invertebrates, such as insects and isopoda, and did not utilize vertebrates, such as fish and anuran larvae (Table 1). From a preliminary survey, there were a number of loaches, *Misgurnus anguillicaudatus*, and tadpoles in the study sites; however, no *C. brevis* larvae ate these vertebrate animals. Like another dytiscid beetle, *Ilybius*, *Rhantus*, and *Agabus* (Lundkvist et al., 2003), a 1st instar *C. brevis* larva is a potential natural predator of Japanese encephalitis vector mosquito *Culex tritaeniorhynchus*, because 1st instar larvae ate mosquito larvae, *Cx. mimeticus*, at this study site. On the other hand, 2nd and 3rd instar consumed higher order insect predators in the food web, such as Odonata nymphs and backswimmers.

From the rearing experiment, all *C. brevis* larvae provided with tadpoles died. Individuals in the Odonata nymph treatment had longer total body length than those in the tadpole-Odonata nymph mixture treatment (Fig. 4a) but the larval period was almost the same (Table 2). In the tadpole-Odonata nymph mixture treatment, the number of Odonata nymphs consumed was more than the number of tadpoles for all instars (Fig. 4b). Although larvae of *C. brevis* could search for and eat motionless Odonata nympha, all larvae died from starvation after being supplied with only motionless tadpoles. These results suggest that *C. brevis* larvae do not consume tadpoles not because they cannot capture them, but because they dislike and/or cannot recognize tadpoles as prey animals. Thus, larvae in the tadpole-Odonata nymph mixture treatment (3 tadpoles and 3 Odonata nymphs were supplied) ate only half the amount of prey animals of larvae in the Odonata nymph treatment (6 Odonata nymphs were supplied). This may have induced the differences in adult body length between the two treatments. Interestingly, larvae of *C. brevis* did not eat tadpoles, although the larvae of large-bodied diving beetles, *Dytiscus* beetles and *Cybister japonicus*, feed on anuran larvae (Young, 1967; Brodie and Formanowicz, 1983; Johansson and Nilsson, 1992; Inoda and Kamimura, 2004; Ohba, 2009). The different feeding habits might be attributable to differences in the digestive enzymes between the species, as identified previously in predatory belostomatid bugs (Swart et al., 2006).

In conclusion, contrary to the views expressed in previous commentaries (e.g., Ichikawa, 1984, 2007; Tsuzuki et al., 1999; Uchiyama, 2005), *C. brevis* larvae preyed mainly on invertebrate animals but did not eat vertebrate animals. These results strongly suggested that environments with abundant aquatic invertebrates were favorable for maintaining the population of *C. brevis*.

ACKNOWLEDGEMENTS

I wish to acknowledge the valuable comments of and discussion with Dr. Fusao Nakasui, Dr. Takahisa Miyatake, Dr. Kenji Matsuura, Dr. Hiroshi Sajo, Maya Fukushima, and Yuuki Kamite. I thank Dr. Yukiko Higa for identifying the *Culex* larvae. I also thank the owners of the rice fields at the study sites who kindly permitted this field work. This study was partly supported by a grant from the Fujifilm Green Fund.

REFERENCES


