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OHBA:

Diet of *C. japonicus* larvae

Population Ecology

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Ontogenetic Dietary Shift in the Larvae of *Cybister japonicus* (Coleoptera: Dytiscidae) in Japanese Rice Fields

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Abstract A number of fragmentary reports suggest that the endangered diving beetle *Cybister japonicus* larvae feed on tadpoles, fish, and aquatic insects. However, no quantitative study on the feeding habits of *C. japonicus* larvae has been reported. In the present study, field observations and rearing experiments were carried out in order to reveal the feeding ecology of *C. japonicus* larvae. Unlike previous commentaries, the 1st and 2nd instar larvae of *C. japonicus* preyed on insects, mainly Odonata nymphs and *Notonecta triguttata* irrespective of prey availability, but did not eat vertebrates such as tadpoles and fish in the field. On the contrary, the 3rd instar larvae fed on both insects and vertebrates. Rearing experiments revealed that the number of Odonata nymphs consumed was significantly more than the number of tadpoles consumed by the 1st and 2nd instars but 3rd instar larvae ate both the Odonata nymphs and tadpoles in the tadpole-Odonata nymph mixture experiment. The total body lengths of *C. japonicus* new adults in the Odonata nymph and tadpole-Odonata nymph mixture treatments were statistically equal. These results suggested that the 1st and 2nd instar larvae of *C. japonicus* prey mainly on insects and do not eat vertebrate animals (insectivore), whereas the 3rd instar larvae fed on both insects and vertebrates (generalist).

KEY WORDS diving beetle, Odonata, Anura, fish, predator

Introduction

Predatory diving beetles (Dytiscidae: Coleoptera) prey upon various dietary items such as cladocerans, insects, amphibians, and fish (Bay 1974, White and Brigham 1996). *Cybister japonicus* Sharp, the largest species of Japanese diving beetles (33-42 mm in body length), is distributed in the Korean Peninsula, Taiwan, China, Siberia and Japan excluding the Ryukyu islands (Mori and Kitayama 2002). The numbers of *C. japonicus* are declining in most regions of Japan, and this species is designated in the *Red Data List* of species in 45 of 47 prefectures of Japan (Japan Environment Agency 2000, Association of Wildlife Research and EnVision 2007). The populations of *C. japonicus* became extinct in Chiba and Kanagawa, central Japan (Nishihara et al. 2006). Contributing factors, such as decreasing amounts of suitable aquatic habitats including the abandonment of rice paddies, water pollution, pesticide application, and invasion by alien species are of great concern (Japan Environment Agency 2000, Nishihara et al. 2006). In addition, the population size of predatory invertebrates is limited by its food resources, as for any other predatory insect (e.g., Lenki 1984, Pearson and Knisley 1985, Juliano 1986). Thus, understanding of the trophic ecology of all life stage is needed to support an insect conservation program.

Cybister japonicus lives in rice paddy water systems, and they reproduce from

May to July in rice fields in Shimane Prefecture, western Japan (Saijo 2001). Saijo (2001) discussed that the reason why *C. japonicus* reproduces in rice fields is the abundant food resources present. A number of fragmentary reports (e.g., Ichikawa 1984, Tsuzuki et al. 1999, Uchiyama 2005, Ichikawa 2007) suggest that *C. japonicus* larvae feed on tadpoles, fish, and aquatic insects. However, no quantitative study on the feeding habits of *C. japonicus* larvae has been reported. In order to reveal the feeding ecology of *C. japonicus* larvae, field observations and rearing experiments were carried out.

Materials and Methods

Study site. To investigate the dietary items of *C. japonicus* larvae, field censuses were carried out in a rice paddy water system in eastern Shimane from 26 April to 27 August in 2007. The field censuses were weekly from April to July and biweekly during August. Censuses were conducted along the ridges around rice paddy water systems such as rice fields. Three ditches (600, 500, and 150 m^2) were set up as census plots in which to investigate the diet of *C. japonicus* and the occurrence frequencies of *C. japonicus* and potential prey. Rice fields were surrounded by a ridge covered with weeds, making a small convenient footpath that reduced site disturbance between adjoining rice fields.

The rice fields are filled with water to depths of 5–15 cm. The water levels in all rice fields were maintained from mid-May to mid-June (irrigation period). In mid-June, the water was drained from the field, and the rice field continued draining for a few weeks, eventually becoming fully drained, with the ground exposed to the sun (drainage period). Nevertheless, the ditch water remained at 3–5 cm depth, even during the drainage period. These water management practices have been described in detail by Saijo (2001).

Occurrence frequency of prey animals. To evaluate the food types available to *C. japonicus* larvae, the relative abundance of prey was investigated during the daytime (1200–1700 h). In consideration of the differences in relative abundance of prey by different sampling methods, both sweeping and quadrat were adopted in this study. A 3-mm-mesh D-frame dipnet (28 cm wide) was pulled 5 times for 50 cm along the bottom of the ditches (hereafter referred to as the sweeping). This procedure was replicated 4 times within one day. Simultaneously, 5 quadrats (see Krebs 2001, 30 cm × 15 cm mouth opening, 20 cm height, 1 mm mesh) were used in order to compare with the data estimated by the sweeping method. The quadrats were established in the ditch, and then all animals were caught using a 500 μm mesh dipnet (10 cm × 4 cm mouth

opening). The sweeping in the quadrat was finished if no animals were captured during the 5 sweepings. After this survey, the prey was released immediately back into the ditch. These censuses were conducted on the same day. Two-way multiple analysis of variance (MANOVA), with “method” and “day” as main factors, was used to compare the species composition (Odonata nymph, Notonectidae, mosquito larva, tadpole and fish) estimated by two sampling methods (sweeping and quadrat) during the research periods.

Frequency of occurrence of *C. japonicus* and prey animals. Beetle individuals were observed directly in the field using a flashlight (11,000 lx) from 2000 to 0200. In the daytime, the observation for beetle was difficult because of the reflection of sunlight on the water surface. The flashlight did not interfere with the foraging behavior of beetle larvae as they did not stop feeding or ambushing prey (S. Ohba, unpublished data). On the contrary, it was impossible to observe the beetle adults because they disappeared into the water immediately when investigator approached. When beetle larvae were found, it was noted if prey was held in their mandibles. The body width of the prey was measured as an index of prey body size and the head width of the *C. japonicus* larva was measured using calipers. The *C. japonicus* larvae were assigned to instars based on

their head width data obtained from preliminary survey (S. Ohba, unpubl. data) as follows: first instar, 2.4–2.9 mm; second instar, 4.1–4.4 mm; and third instar, 5.9–8.6 mm. The type of prey held in the mandibles was recorded as a dietary item and preserved in 70% ethyl alcohol for later identification. After measurement, the *C. japonicus* larvae were released immediately back into the ditch. Spearman's rank correlation coefficient was used to evaluate the association between the head widths of *C. japonicus* larvae and the body widths of their prey (n = 14).

Rearing experiment. Five male and five female *C. japonicus* adults were collected as breeding stock from an irrigation pond in the eastern Shimane, Japan, in April 2008, and kept in an aquarium (55 cm × 40 cm mouth opening, 35cm height) maintained at 27.4 ± 0.11°C (S.E.) water temperature and with a 16L:8D light cycle. River gravel was laid on the bottom of the aquarium in a 20 cm thick layer, and dechlorinated tap water was added over the sand surface to a depth of 15 cm. 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.

First and 2nd instar larvae were reared individually in a plastic container (6 cm

× 6 cm mouth opening, 5 cm height) with one hole (5-mm diameter) in the bottom, and the tops were covered with a plastic board. The bottom hole in each plastic container was covered with net (3 mm mesh). Third instar larvae were transferred to a plastic cage (10 cm diameter × 10 cm height). Both the plastic container and cage were laid on a 1 cm layer of river gavel. To prevent the water quality deteriorating drastically, all of the plastic containers and cage were placed in a large aquarium (63.5 cm × 43.9 cm × 22.6 cm) kept at 29.3 ± 0.07°C (S.E.) water temperature with a 16L:8D light cycle. The aquarium was filled with water to a depth of 15 cm, and aerated with an per air pump and air stone (Ohba 2008). All of the plastic containers were fixed within the aquarium to keep the water depths at 3 for the 1st and 2nd instar and 5 cm for 3rd instar, respectively.

Experiments were conducted separately for three prey treatments: tadpole, Odonata nymph and a tadpole-Odonata nymph mixture. But, the tadpole treatment was not included because it was confirmed by preliminary experiments that first instar larvae (n = 4) could not grow when fed on tadpoles (*Hyla japonica* and/or *Rana nigromaculata*). Because it is known that some insect predators can get high performance when they are provided mixture prey animals (e.g., Sonoda et al. 1992), two prey treatments, the Odonata nymph and the tadpole-Odonata nymph mixture

treatment were conducted. Prey animals in this study were collected from rice fields and irrigation ponds in the field. In the tadpole-Odonata nymph mixture treatment, small tadpoles for 1st instar (<10 mm in SVL), medium tadpoles for 2nd instar (10-25 mm), and large tadpoles for 3rd instar (26-50 mm) of the pond frog *R. nigromaculata* were provided. In the Odonata nymph and tadpole-Odonata nymph mixture treatment, small damselfly nymphs for 1st instar (Platycnemididae: *Copera* spp. and Lestidae: *Lestes* spp., <15 mm), medium damselfly nymphs for 2nd instar (same species, 15-20 mm), and large dragonfly nymphs for 3rd instar (Libellulidae: *Orthetrum albistylum speciosum* Uhler, *Sympetrum frequens* Selys, *S. infuscatum* Selys; Aeshnidae: *Planaeschnu milnei* Selys, and *Anax parthenope* Brauer 20–30 mm) were provided. The density of prey in each plastic container was kept constant (6 Odonata nymphs in the Odonata treatment, and 3 tadpoles and 3 Odonata nymphs in the tadpole-Odonata nymph mixture treatment). The prey density levels were set to supply enough food for *C. japonicus* 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 the containers. Third instar larva that did not eat the prey within one hour after it was provided were moved to a cup (10 cm diameter × 10 cm height) filled with peat moss

for pupation. The day 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 measured for their total length using calipers. The Odonata nymph and tadpole-Odonata nymph mixture treatments were replicated 8 times each. Data from dead individuals, 1 in the Odonata nymph and 2 in the tadpole-Odonata nymph mixture treatments, were excluded from the analysis. One individual in the Odonata nymph treatment was excluded from the measurement and analysis of adult body length because of failure adult eclosion. After all field censuses were finished, all beetle adults were released in the irrigation pond in which beetles were captured.

To evaluate the effect of the prey on the total body length of new adults, one-way analysis of variance (ANOVA) with prey (Odonata nymphs, and tadpole–Odonata nymph mixture) was performed. The larval period of *C. japonicus* in the two prey treatments was compared using repeated-measures one-way ANOVA, with prey (Odonata nymph, or tadpole-Odonata nymph) as the between-subject factor 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 =0.31), significance levels for within-subject effects were not corrected using Greenhouse–Geisser for the degrees of freedom (see Quinn and Keough 2002).

Log_{10} transformations for exact values were made in order to standardize and normalize variances, if necessary to satisfy the assumptions of the ANOVA model. Statistical significance was set at 0.05. All statistical tests were conducted using JMP software (JMP version 7.0, SAS Institute 2007).

Diet selection. To reveal the diet selection of *C. japonicus* larvae, the number of each prey item consumed in the tadpole-Odonata nymph mixture treatment was recorded for each larval instar. Paired *t*-test was used to compare the number of prey consumed between tadpoles and Odonata nymphs for each larval instar.

Results

Frequency of occurrence of *C. japonicus* larvae and prey animals. First and second instar larvae of *C. japonicus* appeared from mid-May to mid-June and mid-May to early July, respectively (Fig. 1). The third instar larvae appeared from June to mid-July. The frequencies of prey animals were estimated by two methods (sweeping and quadrat). The two-way MANOVA on the species composition revealed significant "method" ($F_{5, 452} = 80.6$, $P < 0.001$ for Log_{10} $(x + 1)$ transformed data), "day" (Roy's Greatest Root; $F_{13, 456} = 80.6$, $P < 0.001$) and "method-by-day" interaction effects (Roy's Greatest

Root; $F_{13, 456} = 17.7$, $P < 0.001$). The relative abundances of tadpoles, Odonata nymphs (mainly *Orthetrum* spp. and *Sympetrum* spp.) and *Notonecta triguttata* collected by five sweeps were greater than those by the quadrat method, but their occurrence periods were almost the same between the two methods. Tadpoles (*Hyla japonicus*, *Rana nigromaculata*, and *Rhacophorus schlegelii*) were abundant in June; as the season progressed, they became frogs and their numbers declined. The abundance of fish increased gradually from June to July. The observed fish species were rice fish, *Oryzias latipes* and loach, *Misgurnus anguillicaudatus*. In contrast, Odonata nymphs, *N. triguttata* and mosquito (Culicidae) larvae were present at a low density throughout the season. Only one mosquito larva was captured by the quadrat method on 22 May but no mosquito larvae were captured by five sweeps throughout the season.

Prey composition of *C. japonicus* larvae. There was a significant positive correlation between the head width of *C. japonicus* larva and their prey ($rs = 0.49$, $P = 0.04$, $n = 14$, Spearman's rank correlation coefficient). First and 2nd instar of *C. japonicus* larvae fed on insects, mainly Odonata nymph and *N. triguttata*, and did not prey on vertebrates such as fish and anuran larvae in the field (Table 1). On the other hand, third instar fed on insects, including mainly Odonata nymphs and Coleoptera larvae,

and vertebrates such as *Rana nigromaculata* tadpoles and *Misgurnus anguillicaudatus*.

Effect of diet. From the rearing experiment, the effects of two prey diets, Odonata nymph and the tadpole-Odonata nymph mixture, on the development of *C. japonicus* larvae were evaluated by the total body length of new adults. A one-way ANOVA revealed that the prey effect was not significant ($F_{1, 10} = 0.20$, $P = 0.66$).

For the larval period, repeated-measures one-way ANOVA revealed that the larval stage effect was significant but the prey and prey-by-larval stage interaction effects were not significant (prey: $F_{1, 11} << 0.001$, $P = 0.95$; larval stage: $F_{2, 22} = 124$, $P < 0.001$; larval stage*prey: $F_{2, 22} = 1.80$, $P = 0.19$ for log-transformed data). The larval period of 3rd instar tended to be longer than those of the 1st and 2nd instars, irrespective of prey treatment (Table 2).

Diet selection. In the tadpole-Odonata nymph mixture treatment, the number of Odonata nymphs consumed was significantly more than the number of tadpoles consumed by 1st and 2nd instar larvae (Paired *t*-test, 1st: $t_5 = 9.13$, $P < 0.001$; 2nd: $t_5 = 5.77$, $P < 0.001$ for log-transformed data; Fig. 2). However, third instar larvae consumed both prey animals almost the same ($t_5 = 0.95$, $P = 387$).

Discussion

The body size of prey animals increased as the larvae of *C. japonicus* grew, as for any other predatory insect (Cloarec 1992, Perez Goodwyn 2001, Ohba et al. 2008). The emergence of 1st and 2nd instar larvae of *C. japonicus* seemed to coincide with the appearance period of Odonata nymphs and tadpoles (Fig. 1). However, 1st and 2nd instar larvae fed on insects and did not utilize vertebrates such as fish and tadpoles (Table 1). On the contrary, third instar larvae appeared during the period that tadpoles and fish became abundant (Fig. 1), and fed on Odonata nymphs, tadpoles, and fish (Table 1). The larvae of some dytiscid species are regarded as effective predators of mosquito larvae (Bay 1974, Berman et al. 2000, Lundkvist et al. 2003) but *C. japonicus* larva did not feed on mosquito larva. Mosquito larva might be either too small for *C. japonicus* larva or too low density in this study site.

In the tadpole-Odonata nymph mixture treatment in the rearing experiment, the number of Odonata nymphs consumed was more than that of tadpoles for 1st and 2nd instar larvae (Fig. 2). On the other hand, 3rd instar larvae fed on the both Odonata nymphs and tadpoles. These results were in accordance with the field observation

shown in Table 1. Unfortunately, the sample size of 2nd instar larvae in the field observation was too small (n = 2), but the results of the rearing experiment in 2nd instar larvae may compensate for the field observation; second instar larvae preferred insects to the tadpoles. The total body length of adults reared in the Odonata nymph treatment and those in the tadpole-Odonata nymph mixture treatment were almost the same. This reason might be that 3rd instar larvae could eat both the Odonata nymphs and tadpoles (Fig. 2).

Interestingly, all larval stages of the congeneric *C. brevis* larvae fed on insects and did not utilize vertebrates such as fish and tadpoles in the present study site (Ohba S. unpublished data). Although the reason why the dietary items were different between *C. japonicus* and *C. brevis* was not known, this may be induced by the differences in the head width between the two species (2.4-8.6 mm for *C. japonicus*, 1.4-4.3 mm for *C. brevis* in the head width of larvae) (Ohba S. unpublished data). Or, the different feeding habits might be attributable to differences in the digestive enzymes between the two species, as identified previously in predatory belostomatid bugs (Swart et al. 2006).

In conclusion, unlike in previous commentaries (e.g., Ichikawa 1984, Tsuzuki et al. 1999, Uchiyama 2005, Ichikawa 2007), the 1st and 2nd instar larvae of *C. japonicus* were found to prey mainly on insects and did not eat vertebrate animals

(insectivore) in the field. On the contrary, the 3rd instar larvae fed on both insects and vertebrates (generalist). These results suggested strongly that environments with abundant aquatic insects and vertebrates were favorable for maintaining the population of *C. japonicus*.

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Figure Legend

Fig. 1. Seasonal changes in the frequency of *C. japonicus* larvae and prey animals. Data in the prey animals are mean ± S.E.

Fig. 2. The number of tadpoles and Odonata nymphs consumed by *Cybister japonicus* larvae. Data are mean + S.E. *$P < 0.05$, Paired *t*-test.

Table 1. The list and its percentage of dietary items consumed by *Cybister japonicus* larvae in the field.

	First instar (N = 5)	Second instar (N = 2)	Third instar (N=14)
Insect			
Odonata			
Aeshnidae nymph	-	-	7.1
Libellulidae nymph	20.0	100.0	14.3
Damselfly nymph	20.0	-	7.1
Heteroptera			
Notonecta triguttata	40.0	-	-
Coleoptera			
Cybister japonicus larva	-	-	7.1
Hyphydrus japonicus larva	-	-	14.3
Trichoptera			
Unknown	20.0	-	-
Orthoptera			
Gryllotalpa orientalis	-	-	7.1
Amphibia			
Rana nigromaculata larva (tadpole)	-	-	28.6
Fish			
Misgurnus anguillicaudatus	-	-	14.3
Total	100.0	100.0	100.0

Table 2. The larval period in each instar of *Cybister japonicus* in the rearing experiment

		Larval instar (average days ± SE)		
Treatment	*n*	1st	2nd	3rd
Odonata nymph	7	5.14±0.14	5.90±0.26	9.70±0.36
Tadpole-Odonata nymph mixture	6	5.00±0.37	5.50± 0.37	10.80±0.54

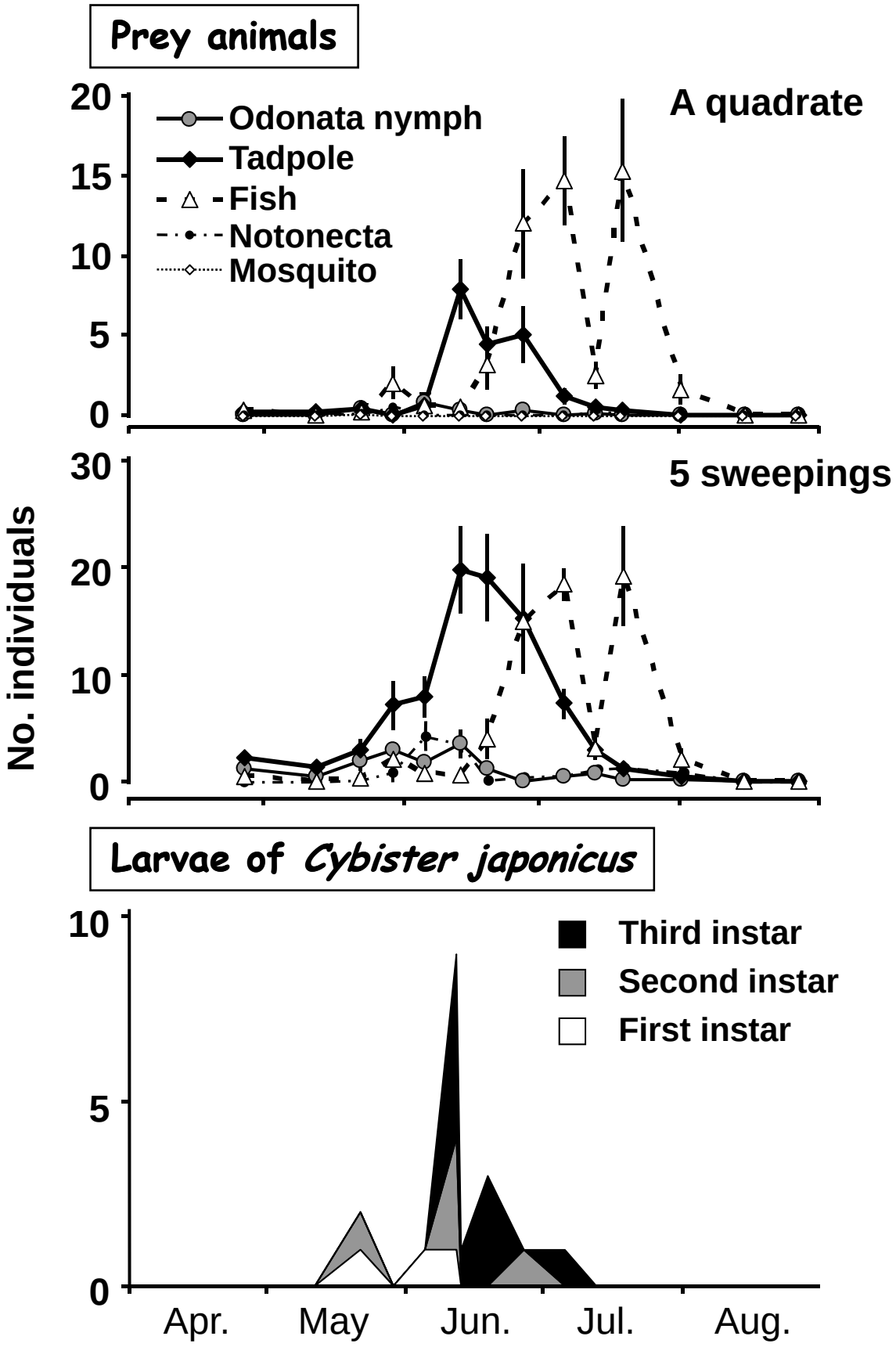

Figure 1. Ohba

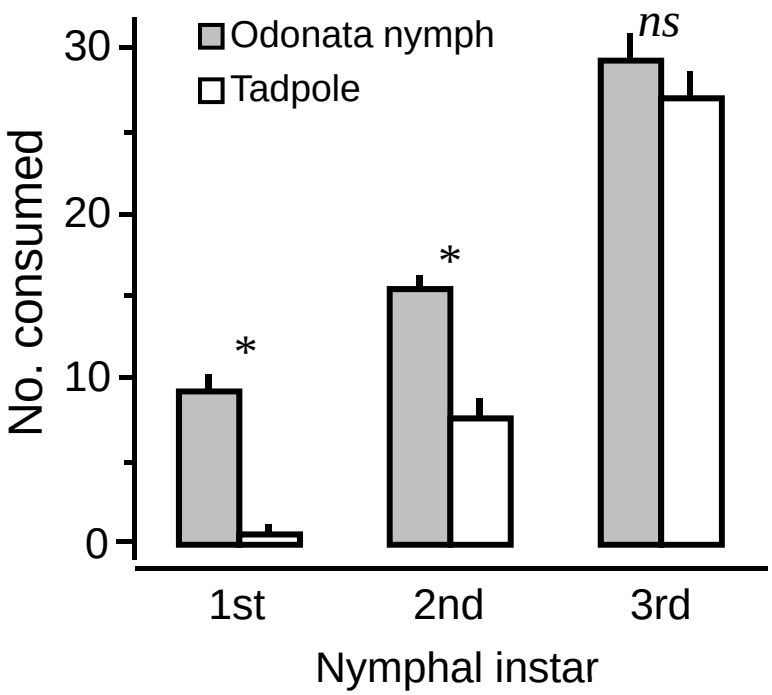

Figure 2. Ohba

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