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Source: Current Herpetology, 38(2) : 115-121

Published By: The Herpetological Society of Japan

URL: https://doi.org/10.5358/hsj.38.115

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Current Herpetology 38(2): 115–121, August 2019
doi 10.5358/hsj.38.115

Larval Prey Preference of Pond-breeding salamander *Hynobius tokyoensis* Living in a Stream

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Abstract: Habitats for pond-breeding salamanders are declining as a result of land development and management abandonment. In that context, a pond-breeding salamander *Hynobius tokyoensis* has recently been known to breed in small streams. However, little is known about the ecology of pond-breeding salamander larvae, such as food, in small streams. Here, to gain insight into foraging ecology of *Hynobius tokyoensis* larvae in small steams, we investigated the potential prey items in nine small streams running through secondary forests in Hachiouji City, Tokyo, Japan. In addition, we hypothesized that *H. tokyoensis* larvae preferentially predate active prey invertebrates over less-active prey because they are generalist, sit-and-wait predators. To test this hypothesis, we experimentally examined predation frequencies by *H. tokyoensis* larvae on the two major arthropod species (isopods and Plecoptera larvae) and activity (i.e., movement frequencies and distances) of the potential prey. As a result, isopods (*Asellus hilgendorfii*) and Plecoptera (*Nemouridae* larvae) are the two dominant aquatic invertebrates in small streams inhabited by *H. tokyoensis*, larval salamanders consumed more isopods than Plecoptera larvae and isopods are more active than Plecoptera larvae. Taken together, we concluded that isopods should be the main prey for salamander larvae inhabiting small streams due to their high abundance and conspicuous activities.

Key words: Salamander; *Hynobius tokyoensis*; Larval diet; Prey preference

INTRODUCTION

Amphibians are the most endangered vertebrate taxonomic group worldwide (Stuart et al., 2004). In Japan, pond-breeding salamanders are at especially high risk of extinction (Matsui, 1996), with 5 of 10 species on the IUCN Red List listed as threatened species (International Union for Conservation of Nature, 2017). This can be attributed primarily to the dramatic decline of suitable aquatic habitats for breeding and larval growth, such as ponds and rice paddy fields in small valleys in hilly or lowland mountain areas, as a result of land development and management abandonment (Matsui, 1996; Kusano et al., 2014). However, one species of pond-breeding salamanders, *Hynobius tokyoensis*, has recently been reported to breed in small streams (Ihara, 2002; Tempaku

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et al., 2011). This type of habitat is thought to compensate for the loss of lentic habitats (Tempaku et al., 2011), and is becoming more important, given the ongoing decline of lentic habitats. Information about the major prey species of salamander larvae inhabiting small streams could be useful for prioritizing habitats for conservation and restoration, as populations of some salamander species are known to be limited by prey availability (Bernardo and Agosta, 2003). However, little is known about the ecology of the larvae of pond-breeding salamander in small streams.

Salamander larvae are expected to feed mainly on dominant aquatic invertebrate species as generalist predators (Kusano and Kawakami, 1999; Huang and Sih, 1991a). Some research has shown that there is a difference in main prey items between stream-breeding and pond-breeding salamanders. Stream-breeding salamanders such as *Hynobius boulengeri* and *Desmognathus quadramaculatus* feed primarily on aquatic larvae of Plecoptera, Ephemeroptera, Trichoptera species (Parker, 1994; Nishikawa and Matsui, 2008; Trice et al., 2015), and isopods (Huang and Sih, 1991b; Smith and Petranka, 1987). On the other hand, pond-breeding salamanders feed mainly on midges, zooplankton (Kusano and Kawakami, 1999; Smith and Petranka, 1987) and conspecific salamander larvae (Kusano, 1981; Kusano et al., 1985; Kuwabara, 1978). This difference is probably due to the difference in the community composition of aquatic invertebrates arising from the characteristics of the physical environments in streams and ponds (Nussbaum, 1985; Parker, 1994). Small stream habitats used by *H. tokyoensis* larvae are small streams flowing through lowland mountain areas and abandoned rice fields. Species compositions of aquatic invertebrates would thus differ between such habitats and pond habitats, but little is known about the species composition of prey items in small steam habitats. It is worth noting here that salamander larvae are sit-and-wait predators and therefore feed primarily on frequently moving animals. It is essential to consider this behavior for examining important prey items.

We assumed that the main prey of *H. tokyoensis* larvae in small streams are aquatic invertebrate species. Under this assumption, we investigated the abundance of aquatic invertebrates in small stream to estimate potential prey items in terms of their abundance. In addition, we hypothesized that larval *H. tokyoensis* preferentially consume the invertebrates that actively move than those which are less active. To test this hypothesis, we experimentally compared activity (i.e., movement frequency and distance) and frequency of being consumed by *H. tokyoensis* larvae between the two species of abundant invertebrates observed in the small streams (i.e., isopods [*Asellus hilgendorfii*] and Plecoptera [Nemouridae larvae], see Results).

Materials and Methods

Study organism

Hynobius tokyoensis is a Red List species endemic to Japan (International Union for Conservation of Nature, 2017). To avoid deleterious effects on this threatened species, we did not analyze stomach contents, and instead employed indirect methods to identify important prey items in a natural setting.

This species is distributed mainly in hilly areas in the Kanto region, eastern Japan, and breeds in ponds or rice fields (Kusano, 1980). After metamorphosis, adult salamanders inhabit the forest floor near aquatic breeding habitats (Kusano, 1980). The breeding season ranges from February to April, lasting for two to three months (Kusano, 1980). Embryonic development takes three weeks, and larvae spend three months in the water after hatching. Metamorphosis takes place in August and September (Kusano, 1981).

Survey of aquatic organisms

Our research sites were small streams running through secondary forests in Hachiouji City, Tokyo (35°41'N, 139°16'E).

The mean length of each stream ranges from 150 m to 500 m. The mean width of streams ranges from 30 cm to 90 cm. The elevation of this site is approximately 200 m. We surveyed nine streams inhabited by *H. tokyoensis* larvae in August 2017 to investigate the community composition of aquatic organisms. In each stream, samples of litter and sediments in an area of 500 cm^2 with a depth of 1 cm was obtained at 15-m intervals along the entire length of the stream. All visible aquatic invertebrates were identified to the order level, and organisms that could not be identified in the field were preserved in a vial with 80% ethanol and identified later in the laboratory.

Experiment 1: Prey preference of salamander larvae

Prey preference was evaluated using three square plastic containers (40 cm×30 cm, 5 cm deep) filled with stream water to a depth of 3 cm. Litter collected from the stream was added to a depth of 1 cm to cover half of the bottom area. We collected three salamanders on the day before this experiment in our research site, and kept them in the same type of plastic containers, as above, in a room exposed to outdoor temperatures in our field (mean temperature: 25.6°C). The three salamander larvae were at a late stage of development, with a mean total length of 42 mm (41–43 mm). For prey, Nemouridae larvae and *A. hilgendorfii* were used because these taxa were dominant in the field (see Results). We collected these prey species from our research site, on the same day with salamander larvae, and kept them in the same type of plastic containers with fallen leaves in outdoor temperatures. 20, 19, 18 individuals from each of Nemouridae larvae and *A. hilgendorfii* were introduced into each of three containers. These numbers were well within the range of natural densities observed in our field survey. The reason for having equal numbers of individuals between prey species in a container is to control the potential encounter rates with salamander larvae. According to the allometric equation of *H. tokyoensis* (Kusano et al., 1985), the mean mouth width of three salamander larvae was approximately 5.3 mm (SD=0.12). As the body-widths of the two prey species used for the experiment were both approximately 2 mm, these prey were well within the range of potential prey of the salamander larvae. After 5 minutes, when prey settled, a single salamander larva was introduced into each container. The experiment was terminated after approximately 24 hours, and the number of each prey species remaining in containers was counted. As no prey died in 24 hours in containers in preliminary observations, all individuals disappeared could be regarded as mortality due to salamander predation. This experiment was conducted in a room exposed to outdoor temperatures and day length conditions in our field in early August.

Experiment 2: Activity of aquatic prey

Ten and five individuals of *A. hilgendorfii* and Nemouridae larvae, respectively, were introduced into a plastic square container (40 cm×30 cm×5 cm) filled with stream water and litter, as in the Experiment 1. These densities of the two species were similar to those observed in actual streams. A plastic container was placed on a graph paper with 1 mm squares. Two individuals of each prey species were chosen for observations of their activity, the distance travelled by prey arthropods and the prey movement frequencies were measured by eyes for 10 minutes, seven times a day (at 5:30, 7:00, 10:30, 13:40, 17:10, 20:00, and 0:30), and the distances or the frequencies of all movement events combined were used for analysis. This experiment was conducted again in a room exposed to outdoor temperatures and day length conditions in early August.

Statistical analysis

A log-linear model with a three-way contingency table was used to test whether predation rates of salamander larvae differ between prey species (Experiment 1). The three factors

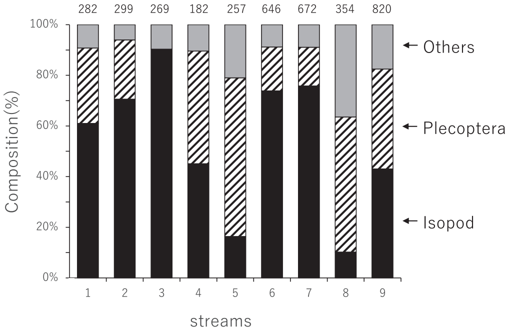

FIG. 1. Taxonomic composition of aquatic invertebrates in nine streams. Numerals are the total aquatic invertebrates captured.

were prey species (P), before/after the experiment (T), and salamander identity (S). Our aim was to test the significance of the two-way P×T interaction, after confirming the non-significance of the three-way interaction (P×T×S). Here, ANOVA was not used for the difference in predation rates because proportion data could not account for sample size.

Next, a two-way analysis of variance was used to evaluate differences in movement distances and frequencies among aquatic prey species (Experiment 2). Both of distances and frequencies were square-root transformed to meet the assumption of normality and homogeneity of variances.

RESULTS

A field survey of aquatic invertebrates demonstrated that *A. hilgendorfii* and Nemouridae larvae account for over 80% of aquatic invertebrates in nine streams; in particular, 60% of aquatic fauna are *A. hilgendorfii* (Fig. 1). The average densities of *A. hilgendorfii* and Nemouridae larvae were 0.01/cm^2 and 0.005/cm^2, respectively.

In the predation experiment (Experiment 1), the number of *A. hilgendorfii* decreased dramatically after the 24 hr exposure to the salamander larvae, while Nemouridae larvae showed little change in abundance (Fig. 2). A log-linear model indicated that the three-way interaction (P×T×S) was not significant (χ^2=0.094, P=0.954), while the two-way interaction of P×T was highly significant (χ^2=17.50, P<0.001). This indicates that there was a significant difference in survivorship due to salamander predation between the two species.

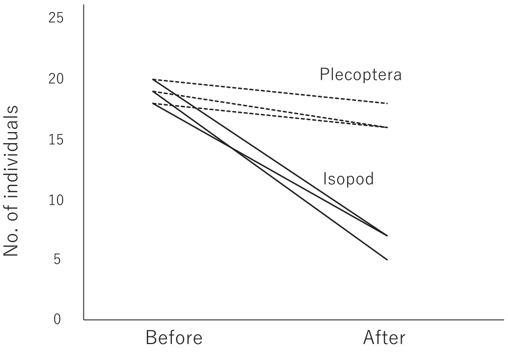

FIG. 2. Comparison of prey abundances before and after 24 hours of the predation experiment. Dotted and solid lines show abundances of Nemouridae larvae and *Asellus hilgendorfii*, respectively.

A two-way ANOVA (Experiment 2) revealed that *A. hilgendorfii* moved significantly longer distances than Nemouridae larvae (F=17.56, df=1, P<0.001) (fig. 3), and also moved more frequently than Nemouridae larvae (F=13.49, df=1, P=0.003) (fig. 3). The distances and frequencies changed with time of a day (F=2.88, df=6, P=0.048) (F=2.610, df=6, P=0.065), although the interaction was not significant (F=1.43, df=6, P=0.272) (F=1.239, df=6, P=0.345).

DISCUSSION

Our results showed that *A. hilgendorfii* and Nemouridae larvae were the two dominant aquatic invertebrates in small streams inhabited by the salamander *H. tokyoensis*. Although this is based on a snapshot survey, the conclusion does not seem to change greatly, as sampling conducted in late May in

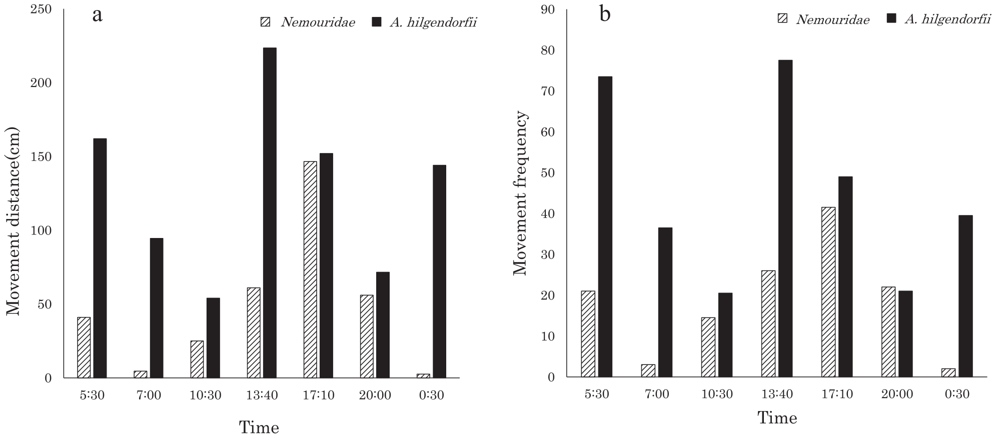

FIG. 3. (a) Mean moving distance of each prey aquatic species in a 10 minutes interval in different times of a day. (b) Mean moving frequency of each prey aquatic species in a 10 minute interval in different times of a day.

the same river system showed a similar pattern, i.e., *A. hilgendorfii* showed the largest abundance (29%), followed by chironomids (19%) and Nemouridae larvae (13%) (Takaho unpublished data). As chironomids are much smaller in body size, *A. hilgendorfii* and Nemouridae larvae appear to be the two major aquatic insects that can be available for larval salamander even in other seasons. Experiment 1 revealed that larval salamanders consumed more *A. hilgendorfii* than Nemouridae larvae. Furthermore, experiment 2 showed that *A. hilgendorfii* are more active than Nemouridae larvae. Taken together, we conclude that *A. hilgendorfii* are the main prey for salamander larvae inhabiting small streams due to their high abundance and conspicuous activities. These results are consistent with those of earlier studies indicating that larval salamanders are predators preferring frequently moving species, as is typical of sit-and-wait predators (Parker, 1994). As we observed no appreciable difference in prey activities between experiment 1 (with salamander larvae) and experiment 2 (without salamander larvae), we consider *A. hilgendorfii* to be more active than Nemouridae larvae, irrespective of the predator presence.

Larvae of *H. tokyoensis* are known to frequently feed on conspecifics (Kusano, 1981). Thus, conspecific larvae may also be an important prey item. As we did not examine stomach contents, we cannot infer the extent of cannibalism. Some research has shown that cannibalism frequently occurs when individuals are confined in enclosures (Reques and Tejedo, 1996; Semlitsch and Reichling, 1989). It is therefore likely that the frequency of cannibalism is determined by the frequency of contact among conspecifics, that is, by larval density (Semlitsch and Reichling, 1989; Wildy et al., 2001). In streams, salamander larvae are sparsely distributed and cannibalism is seldom observed in comparison with ponds (Casper et al., 2015; Reinhardt, 2014). The density of *H. tokyoensis* in our study streams was also low; 10 egg clutches on average along a 150 m length of stream. Thus, cannibalism may be unlikely, and *A. hilgendorfii* appear to be important prey for *H. tokyoensis* in small streams.

The results of our study have implications for the conservation of *H. tokyoensis*. Similar to other salamanders, breeding and larval habitats have been substantially reduced for this species owing to land development (Kusano et al., 2014) and the abandonment of rice paddy fields (Kusano and Kawakami, 1999). Small streams are thought to compensate for the loss of still-water habitats (Tempaku et al., 2011). Given the ongoing decline of lentic habitats, small streams are expected to become more valuable in the near future for the persistence of regional populations of *H. tokyoensis*. Therefore, the identification of streams that should be prioritized for conservation or restoration is an urgent issue. Because survivorship is lowest at the larvae stage in salamanders, determining suitable habitats based on major prey availability at this stage is a promising approach. In this context, the abundance of *A. hilgendorfii* could be used as a proxy for habitat quality for *H. tokyoensis* in streams. It would be interesting to study in the future whether stream-dwelling populations are differentiated in ecological traits, such as feeding morphology, as is known in other salamander species (Nussbaum, 1985). If such a differentiation is genetically determined, stream-dwelling populations need to be given a high conservation priority.

It should be further noted that, in general, salamander breeding sites are spatially linked with surrounding terrestrial environments, as salamander larvae generally habit small water bodies (Corn and Bury, 1989) where the majority of basal resources derive from surrounding forests (Polis et al., 1997). In addition, adult salamanders live on the forest floor for long periods of time (Kusano, 1980). Thus, future work should focus on terrestrial habitat qualities in addition to aquatic habitats to obtain useful information for the integrated habitat management of salamanders.

Acknowledgments

We wish to thank Tamotsu Kusano for providing helpful advices. We also thank Moto Gomi for giving information of salamander habitats.

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Accepted: 19 March 2019