

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

Authors: Takagi, Kaori, and Miyashita, Tadashi Source: Current Herpetology, 38(2) : 115-121 Published By: The Herpetological Society of Japan URL: https://doi.org/10.5358/hsj.38.115

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at <u>www.bioone.org/terms-of-use</u>.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

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

KAORI TAKAGI* AND TADASHI MIYASHITA

School of Agricultural and life Sciences, The University of Tokyo, 1–1–1, Yayoi, Bunkyo-ku, Tokyo 113–8657, JAPAN

Abstract: Habitats for pond-breeding salamanders are declining as a result of land development and management abandonment. In that context, a pondbreeding 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 prev 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 prev 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

^{*} Corresponding author. Tel: +81–3–5841–7543; E-mail address: kaoriyama@es.a.u-tokyo.ac.jp

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 streambreeding and pond-breeding salamanders. Stream-breeding salamanders such as Hynobius boulengeri and Desmognathus quadramaculatus feed primarily on aquatic larvae of Ephemeroptera, Plecoptera. 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 prev 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 prev 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 prev 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 cm² 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 ($40 \text{ cm} \times 30 \text{ cm}$, 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 prev of the salamander larvae. After 5 minutes, when prev 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 prev 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 $(40 \text{ cm} \times 30 \text{ cm} \times 5 \text{ cm})$ 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 prev 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

Terms of Use: https://bioone.org/terms-of-use

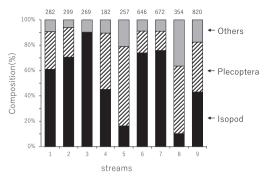


FIG. 1. Taxonomic composition of aquatic invertebrates in nine streams. Numerals are the total aquatic invertebrates captured.

were prey species (P), before/after the experiment (T), and salamander identity (S). Our aim was to test the significance of the two-way $P \times T$ interaction, after confirming the nonsignificance of the three-way interaction ($P \times T \times S$). 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 $0.01/\text{cm}^2$ and $0.005/\text{cm}^2$, 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

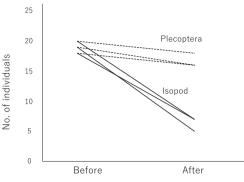


FIG. 2. Comparison of prey abundances before and after 24 hours of the predation experiment. Dotted and solid lines show abundances of Nemouridae larvae and *Asellus hilgendorfii*, respectively.

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.

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

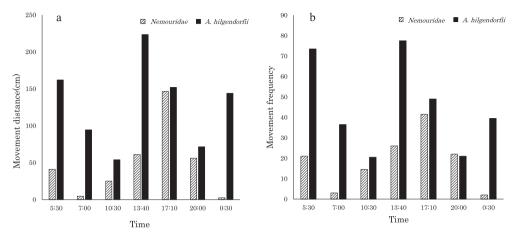


FIG. 3. (a) Mean moving distance of each prey aquatic species in a 10 minutes interval in different times of a day. (b) Mean moving frequency of each prey aquatic species in a 10 minute interval in different times of a day.

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 prev 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 prev 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.

LITERATURE CITED

- BERNARDO, J. AND AGOSTA, S. J. 2003. Determinants of clinal variation in life history of dusky salamanders (*Desmognathus ocoee*): prey abundance and ecological limits on foraging time restrict opportunities for larval growth. *Journal of Zoology* 259: 411–421.
- CASPERS, B. A., STEINFARTZ, S., AND KRAUSE, E. T. 2015. Larval deposition behaviour and maternal investment of females reflect differential habitat adaptation in a genetically diverging salamander population. *Behavioral Ecology* and Sociobiology 69: 407–413.
- CORN, P. S. AND BURY, R. B. 1989. Logging in western Oregon: responses of headwater habitats and stream amphibians. *Forest Ecology and Management* 29: 39–57.
- HUANG, C. AND SIH, A. 1991a. An experimental study on the effects of salamander larvae on isopods in stream pools. *Freshwater Biology* 25: 451–459.
- HUANG, C. AND SIH, A. 1991b. Experimental studies on direct and indirect interactions in a three trophic-level stream system. *Oecologia* 85: 530– 536.
- IHARA, S. 2002. Site selection by *Hynobius tokyoensis* for breeding in a stream. *Current Herpetology* 21: 87–94.
- International Union for Conservation of Nature. 2017. IUCN Red List of Threatened Species. Gland, http://www.iucnredlist.org/ (accessed 15 May 2018)
- KUSANO, T. 1980. Breeding and egg survival of a population of a salamander, *Hynobius nebulosus tokyoensis* Tago. *Researches on Population Ecology* 21: 181–196.
- KUSANO, T. 1981. Growth and survival rate of the larvae of *Hynobius nebulosus tokyoensis* Tago (Amphibia, Hynobiidae). *Researches on Population Ecology* 23: 360–378.
- KUSANO, T., KAWAKAMI, Y., AND MITARAI, N. 2014. Tokyo salamander: Changes in the past ten years. *Tokyo Salamander Research Society*, Tokyo.
- KUSANO, T., KUSANO, H., AND MIYASHITA, K. 1985. Size-related cannibalism among larval *Hynobius nebulosus. Copeia* 1985: 472–476.

- KUSANO, T. AND KAWAKAMI, Y. 1999. Does Hynobius tokyoensis survive? Workshop on the Biology and Conservation of Hynobius tokyoensis. Privately Published, Tokyo. 69 p. (in Japanese)
- KUWABARA, T. 1978. Growth process and diet of larval *Hynobius nigrescens*. Research Report. Niigata Prefectural Education Center 18: 77–84. (in Japanese)
- MATSUI, M. 1996. Natural history of the amphibia. *University of Tokyo* press. Tokyo.
- NUSSBAUM, R. A. 1985. The evolution of parental care in salamanders. Museum of Zoology, University of Michigan, Ann Arbor.
- PARKER, M. S. 1994. Feeding ecology of streamdwelling Pacific giant salamander larvae (*Dicamptodon tenebrosus*). *Copeia* 1994: 705– 718.
- POLIS, G. A., ANDERSON, W. B., AND HOLT, R. D. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology* and Systematics 28: 289–316.
- REINHARDT, T. 2014. New home, new life: the effect of shifts in the habitat choice of salamander larvae on population performance and their effect on pond invertebrate communities. Doctor's thesis. Technical University of Dresden, Freistaat Sachsen.
- REQUES, R. AND TEJEDO, M. 1996. Intraspecific aggressive behaviour in fire salamander larvae (*Salamandra salamandra*): the effects of density and body size. *Herpetological Journal* 6: 15–19.

- SEMLITSCH, R. D. AND REICHLING, S. B. 1989. Density-dependent injury in larval salamanders. *Oecologia* 81: 100–103.
- SMITH, C. K. AND PETRANKA, J. W. 1987. Prey size-distributions and size-specific foraging success of *Ambystoma* larvae. *Oecologia* 71: 239– 244.
- STUART, S. N., CHANSON, J. S., COX, N. A., YOUNG, B. E., RODRIGUES, A. S., FISCHMAN, D. L., AND WALLER, R. W. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783–1786.
- TEMPAKU, M., KANEDA, M., OSAWA, S., AND KATSUNO, T 2011. Distribution and retention of *Hynobius tokyoensis* egg sacs in running water bodies. *Journal of the Japanese Institute of Landscape Architecture* 74: 497–500.
- TRICE, A. E., ROSEMOND, A. D., AND MAERZ, J. C. 2015. Diet composition of two larval headwater stream salamanders and spatial distribution of prey. *Freshwater Biology* 60: 2424–2434.
- WILDY, E. L., CHIVERS, D. P., KIESECKER, J. M., AND BLAUSTEIN, A. R. 2001. The effects of food level and conspecific density on biting and cannibalism in larval long-toed salamanders, *Ambystoma macrodactylum. Oecologia* 128: 202–209.

Accepted: 19 March 2019