Preliminary study of food habits in the Japanese clawed salamander larvae *(Onychodactylus japonicus)* in a mountain brook of the Kiso River system

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Abstract

To evaluate food habits of the Japanese clawed salamander larvae *(Onychodactylus japonicus)*, we examined stomach contents of 22 individuals collected from a natural mountain brook in a tributary of the Kurokawa River in Kiso Fukushima, Nagano Prefecture, central Japan. Their diet composition did not differ between fast and slow current conditions. The diet reflected the natural benthos communities of the brook, in which mayfly nymphs and caddisfly larvae accounted for 70–88%. The salamander larvae selectively foraged on caddisfly and fly larvae in the two current conditions. In the slow current condition, their prey preference was detected in the prey group including terrestrial invertebrates. Our results suggest that the prey items of *O. japonicus* larvae hinge primarily on the natural benthos communities.

Key words: benthic animals, habitat traits, Manly’s Index, prey preference, terrestrial invertebrates

Introduction

Diverse food items found in animal stomachs represent the ability to utilize a wide variety of prey taxa. In fact, knowledge of the feeding habits of aquatic animals, such as aquatic insects, fish and amphibians, can provide useful system-specific information on variation in feeding behavior, food availability, and prey species diversity (e.g., Nakano *et al.,* 1999; Hirai and Matsui, 2000; Matsui *et al.,* 2003; Ohba and Nakasuji, 2006).

Lotic habitats are characterized by heterogeneous physical conditions, even within a local area (Nowell and Jumars, 1984). The action of flowing water is a dominant physical feature of river systems, which mediates physiological processes such as temperature and oxygen availability and influences the distribution and abundance of benthic animals (e.g., Genkai-Kato *et al.,* 2000, 2005). Water flow affects interactions among organisms such as grazer–periphyton (Poff and Ward, 1995; Kuhara *et al.,* 2000) and predator–prey interactions (Hansen *et al.,* 1991).

The Japanese clawed salamander, *Onychodactylus japonicus* (Houttuyn) (Fig. 1), is distributed in Honshu and Shikoku Islands of Japan and is found in montane regions ranging from a few hundred meters to over 2000 m above sea level (Goris and Maeda, 2004). Salamander larvae inhabit lotic environments, such as brooks and streams, for two to three years until metamorphosed (Ogihara and Nakamura, 1974; Hayase and Yamane, 1982). The presence of salamander larvae is a symbol of clear water in aquatic systems. Thus, salamanders are of importance for conservation. In recent years, however, they have decreased in abundance as shown in other amphibian species (Stuart *et al.,* 2004), and are listed as a near threatened species or vulnerable species in some prefectures of Japan (e.g., Sugiyama, 2004).

The dietary items and habitat traits can be closely

Fig. 1 Japanese clawed salamander larvae (*Onychodactylus japonicus*)

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related with the survival of salamander. Stomach contents are one of the most relevant features of the life history of amphibians (Ihara, 1998; Blackburn and Moreau, 2006; Wheeler *et al.,* 2007), although direct observations of foraging behavior in the field are difficult. There is no quantitative or qualitative report on the food items of *O. japonicus* larvae in natural environments. Here, we examined habitat traits and prey compositions in stomachs of *O. japonicus* larvae in a mountain brook of Japan.

1. Methods

A total of 22 salamander larvae were collected on 30 July 2001 from Akashio-zawa Brook (35°52´N, 137°40´E) in Kiso Fukushima, Nagano Prefecture, central Japan (Fig. 2). The brook is a tributary of Kuro-kawa Stream, flowing into the Kiso River system (Fig. 3). The Kiso River system flows finally into the Pacific Ocean through Ise Bay. Sampling of salamander larvae was conducted with a quadrat net (10 × 8 cm, <1-mm mesh) by randomly collecting a stone (loose cobble) from the streambed of the Akashio-zawa Brook. We measured the snout–vent length of collected individuals as an indicator of body size to the nearest 0.01 mm using a digital caliper (Code No. 500-151, Mitutoyo, Kanagawa, Japan). To assess habitat traits with regard to physical conditions, we measured the current velocity and water column depth at each site where a larva was collected. We also measured the surface area of the stone when a larva was collected. Current velocity was measured at 60% depth to estimate the mean current velocity (Hynes, 1970), using a portable current meter (Model CR-7WP, Cosmo-Riken,

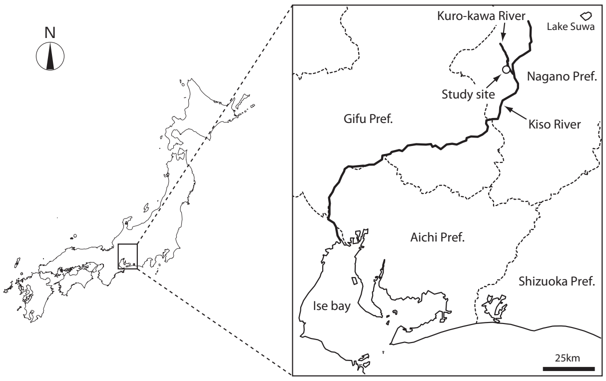

Fig. 2 Study site (open circle) of Akashio-zawa Brook in Kiso Fukushima, Nagano prefecture, and the Kiso River system in central Japan.

Fig. 3 Akashio-zawa Brook (a, b), and Kuro-kawa Stream into which the brook flows (c, d).

Kashihara, Japan). Water temperature in the sampling brook was measured on the sampling day. The collected salamanders were killed immediately by immersion in 5% formalin to preserve the stomach contents. They were then transported to the laboratory of the Kiso Biological Station, Kyoto University. The stomach contents were examined by abdominal dissection under a binocular microscope.

To assess the benthos communities as potential prey items for the salamander larvae in the brook, we collected benthic animals with a quadrat net (15 × 15 cm, <1-mm mesh) at fast and slow current sites (3 replicates at each site). Benthic animals were categorized into mayfly nymphs, caddisfly larvae, stonefly nymphs, fly larvae, and others (e.g., dragonfly nymphs and terrestrial invertebrates). The proportional contribution of each prey category to the total stomach contents or the total benthos communities in the brook was determined for each current condition.

The physical conditions of the microhabitats were compared between two current conditions with t-tests. Animal prey compositions were compared between two current conditions in the field survey and stomach contents using multivariate analysis of variance (MANOVA). Proportional data were arcsine-square-root transformed. The Manly's Index (Chesson, 1978) was used as a measure of prey selectivity:

$$\alpha_i = \frac{r_i / n_i}{\sum_{j=1}^{m} r_j / n_j}, \quad i = 1,2,\ldots,m$$

where r_i and n_i are the proportions in biomass of prey category *i* occurring in stomach contents and field survey, respectively. The total number of prey item categories, *m*, is 5 in this study. This index, which removes bias due to differing availability of prey categories, lies between 0 (never eaten) and 1 (100% eaten). A preference is indicated by values of $\alpha_i > 1/m$ (i.e., 1/5 = 0.20). MANOVA was performed on statistical package SPSS (version 11.0, SPSS, Chicago, IL, USA). Data are presented as means ± SE throughout the text and figures.

2. Results and discussion

Water temperature was 14.0–14.5ºC in the sampling brook. The snout–vent length of the collected 22 larvae was 22.0 ± 0.8 mm, corresponding to 0.10–1.10 (0.40 ± 0.06) g in wet weight according to Takahara *et al*. (2008). Salamander larvae tended to inhabit two distinct environments with regard to current velocity (Fig. 4). The collected larvae were classified into fast (6 individuals) and slow (16 individuals) current groups. The current differed significantly between two groups (Fast: 43.6 ± 2.3 cm/s; Slow: 8.0 ± 1.2 cm/s; t-test, $t_8 = -13.64$, $P < 0.001$), but the snout–vent length did not differ ($t_8 = -0.69$, $P = 0.51$). The surface area of the stone where a larva was collected differed significantly between two current conditions

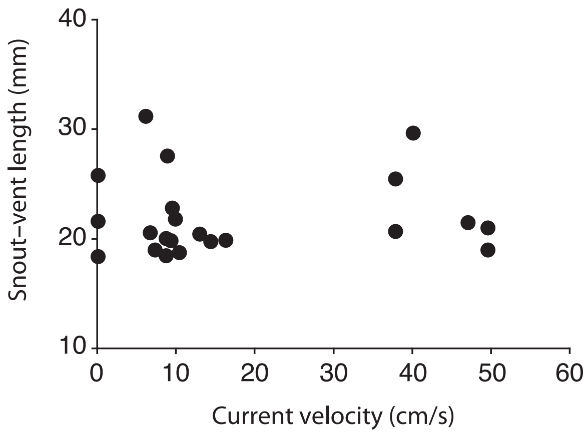

Fig. 4 Relationships between current velocity and snout–vent length of *O. japonicus* larvae.

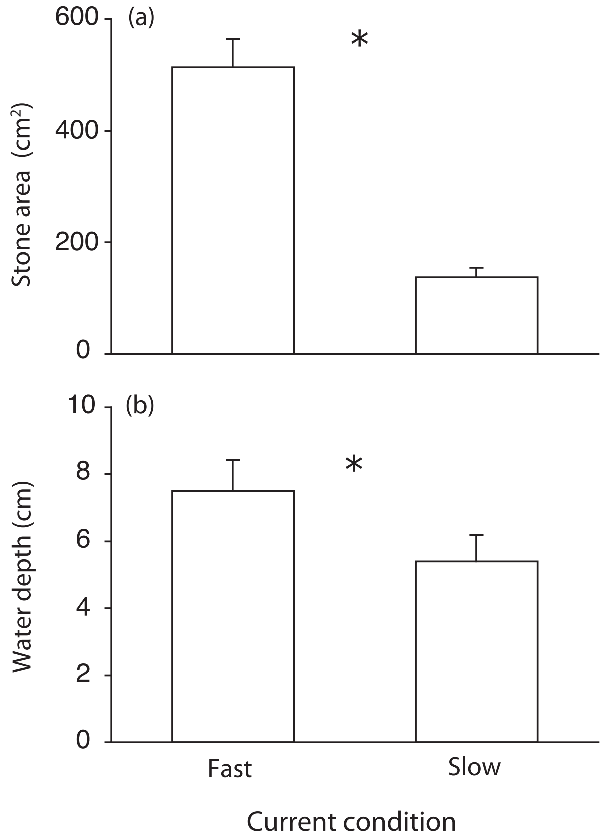

Fig. 5 Surface area of stone (a) and water depth (b) of two current microhabitat conditions of *O. japonicus* larvae. Means ± SE are from 6 and 16 individuals for fast and slow current conditions, respectively. Asterisks denote significant difference between two current conditions (t-test, *P* < 0.05).

(Fast: 513.7 ± 50.5 cm^2, Slow: 137.7 ± 16.6 cm^2; t-test, t_5 = -10.02, $P < 0.001$; Fig. 5a). The water column depth at sites where a larva was present also differed significantly between two current conditions (Fast: 7.5 ± 0.9 cm, Slow: 5.4 ± 0.8 cm; t_8 = 8.31, $P < 0.001$; Fig. 5b).

Field survey showed that the compositions of benthic animals did not differ between fast and slow current conditions (MANOVA, Wilks' lambda = 0.50, $F_{4,1}$ = 0.25, P = 0.89). Mayfly nymphs and caddisfly larvae accounted for 82% (fast) and 70% (slow) of the total benthos communities (Fig. 6a, b). Terrestrial invertebrates (e.g., crane fly; categorized as "Others") were collected only in the slow current condition. The composition of stomach contents did not differ between two current conditions (Wilks' lambda = 0.88, $F_{5,16}$ = 0.42, P = 0.83). The stomach contents were similar to the benthos communities in the brook, in that both mayfly nymphs and caddisfly larvae accounted for 88% (fast) and 70% (slow) in the total stomach contents (Fig. 6a, b). In addition, terrestrial invertebrates (moth larvae, flies, and mites; categorized as "Others") were found from stomachs only in the slow current condition. This is consistent with the results of field survey in that terrestrial invertebrates were found only in the slow current condition.

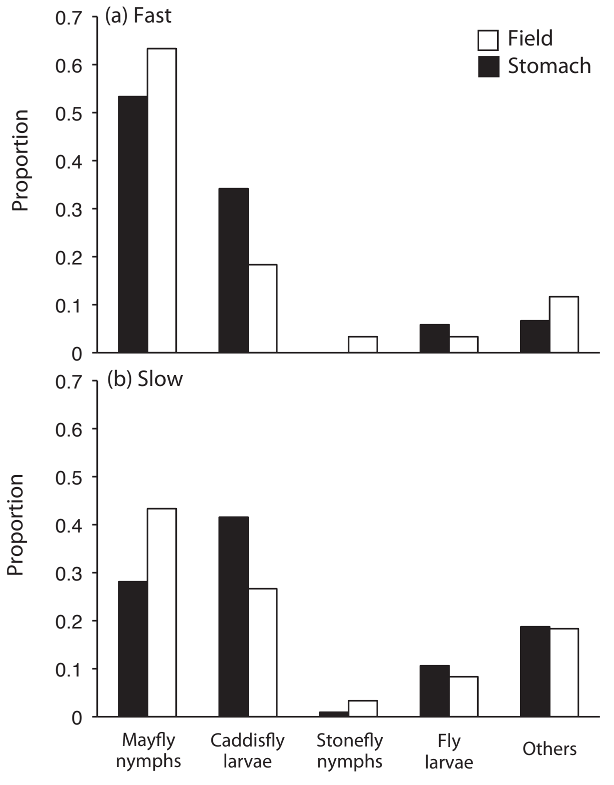

Fig. 6 Proportions of each prey category collected from the field and found in the stomach contents of *O. japonicus* larvae. Means of stomach contents were calculated from 6 and 16 individuals for fast (a) and slow (b) current conditions, respectively.

The Manly's Index showed that *O. japonicus* larvae selectively foraged on caddisfly and fly larvae in the two current conditions (Fig. 7a, b). Despite the high abundance of mayfly nymphs in the field (Fig. 6), feeding preference was not detected in the stomach contents (Fig. 7a, b). Salamander larvae may prefer prey animals with less mobility in natural environments, because caddisfly and fly larvae are less-mobile compared with mayfly nymphs. Salamander larvae selectively foraged prey items of the group "Others" in the slow current condition (Fig. 7b). This preference is due to terrestrial prey items found only in the slow current condition. *Onychodactylus japonicus* may catch occasionally flowing terrestrial insects because of slow current.

Listed in table 1 are the number of salamander larvae that consumed prey items in each category (N), and the proportion of salamander larvae that consumed prey items in each category (R). We also showed the prey animals that were identified to the family level (Table 1). The caddisfly and fly larvae were frequently found from the stomachs (R = 0.68 for caddisfly and R = 0.59 for fly).

Our study on stomach contents suggests that natural benthos communities are of primary importance for the survival of *O. japonicus* larvae. Knowledge of the food habits in the natural environments is necessary for understanding life history of amphibian species (Ihara, 1998; Kohmatsu, 2001; Matsui *et al.,* 2003). It has been reported that the frog *Rana nigromaculata* feed on larger prey animals as it grows (Hirai, 2002). In addition, prey

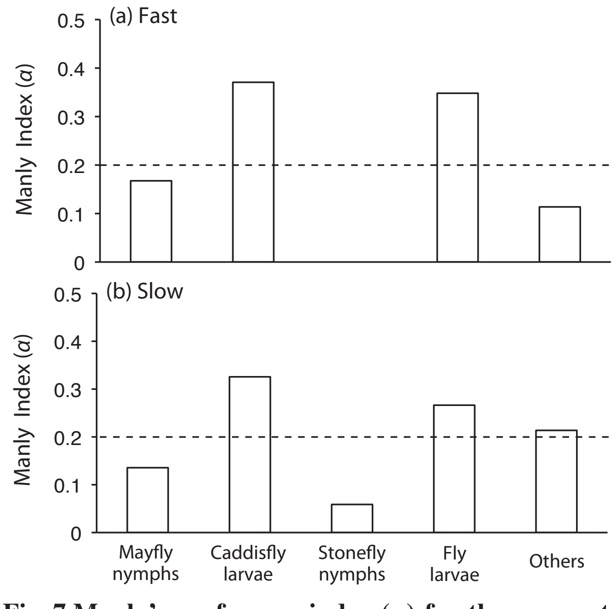

Fig. 7 Manly's preference index (α) for the prey categories of *O. japonicus* larvae in fast (a) and slow (b) current conditions, respectively. The α values above the broken line at 0.2 indicate that the prey is preferred.

Table 1 Summary of stomach contents of 22 salamander *O. japonicus* larvae. N, number of salamander larvae that consumed prey items in the category; R, proportion of salamander larvae that consumed prey items in the category, i.e., R = N/22.

Prey category Order	N	R	Animals that were identified to the family level
Mayfly nymphs			
Ephemeroptera	11	0.50	Heptageniidae (7), Baetidae (2), Ephemerellidae (2), unidentified (4)
Caddisfly larvae			
Trichoptera	15	0.68	Rhyacophilidae (5), Glossosomatidae (2), unidentified (17)
Stonefly nymphs			
Plecoptera	1	0.05	Leuctridae (1)
Fly larvae			
Diptera	13	0.59	Chironomidae (23)
Others			
	8	0.36	Hydrophilidae^{A} (3), Chironomidae^{A} (1), Carabidae^{A} (1), Athericidae^{L} (1), Tipulidae^{A} (1) Lepidoptera^{L}* (1), Acari ^{U}* (1)

Number of prey animals that were identified to the family level is shown in parentheses.
Asterisks (*) indicate prey items that were identified only to the order level in the category "Others".
"A", "L" and "U" indicate adults, larvae (or nymphs), and unknown, respectively.

items for the frog *Hyla japonica* seem to vary seasonally the number and species in natural environments (Hirai and Matsui, 2000). Therefore, such ontogenetic and seasonal changes in the diet of *O. japonicus* larvae need further investigation for their conservation.

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木曽福島の黒川支流に生息するハコネサンショウウオ幼生*Onychodactylus japonicus*の食性に関する予備的調査

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要　旨

2001年7月，長野県木曽福島における黒川支流アカシオ沢に生息するハコネサンショウウオ*Onychodactylus japonicus*の幼生を採集し，食性と生息場所の物理環境について調べた．ハコネサンショウウオ幼生の生息場所は，流速の速いところと遅いところの2つに分かれたが，流速によって胃内容物とアカシオ沢における餌動物群集の組成比に違いはみられなかった．ハコネサンショウウオ幼生は流速の速いところと遅いところの両方でトビケラとハエ目の幼虫を選好し，流速の遅いところでのみ陸生昆虫を含む餌動物のグループを選好していた．ハコネサンショウウオ幼生は，底生動物を主要な餌資源として利用しており，流速が餌の選好性に影響を及ぼしていることが示唆された．

キーワード：餌選好性，底生動物，マンリーの選択性指数，物理環境，陸生昆虫