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[SHORT COMMUNICATION]

Food Habit of the Juvenile of the Japanese Newt *Cynops pyrrhogaster*

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ABSTRACT—The previous study showed that the red coloration of the ventral skin of the Japanese newt *Cynops pyrrhogaster* was associated with the number of carotenoid vesicles and the content of carotenoid in the pigment cell of the skin. To elucidate the mechanism for the red coloration of the skin of the newt, we studied the food habit of the juvenile from the Japanese newt *Cynops pyrrhogaster*. Sixty-two juveniles were collected in Fukue Island in Nagasaki Prefecture from November 2000 to May 2002 and divided into 2 groups according to the snout-vent length (SVL). Over 400 prey animals were obtained from the juveniles by stomach flushing. In the larger group (SVL>30.0mm), Collembola (45.4%) and Acari (12.6%), which are very common species of soil animals, were the prey animals dominant in number. In the group with the smaller SVL (<29.9mm), Collembola (30.4%) and Acari (25.4%) were in number as well. We also studied the food habit of the Japanese clouded salamander, *Hynobius nebulosus*. In the salamander, Doratodesmidae (56.5%) and Amphipoda (13%) were the prey animals dominant in number. Our results, taken together, suggest that the Japanese juvenile *C. pyrrhogaster* does not change its food habit as it grows, and that it eats soil animals common in its habitat. Moreover, the food habit of juvenile *C. pyrrhogaster* differs from that of *H. nebulosus*, although the juveniles of both species live in the same area.

Key words: food habits, newt *Cynops pyrrhogaster*, salamander *H. nebulosus*

INTRODUCTION

The color of the ventral skin of the Japanese newt *C. pyrrhogaster* is creamy at metamorphosis, but it turns red as the animal grows. In amphibians, 3 kinds of pigment cells, i.e., melanophores, xanthophores and iridophores, can be seen in the ventral skin of the juvenile after metamorphosis (Bagnara, 1998). We have shown that adult newts have many carotenoid vesicles in the xanthophores in the red skin (Matsui *et al*., 2002), and that the number of carotenoid vesicles in the pigment cell was very low in the ventral creamy skin at metamorphosis, but increased in the red skin (Matsui *et al*., 2003). The carotenoid content was also very low in the creamy skin of small juveniles, but dramatically high in the red skin of adult newts (Matsui *et al*., 2003). Based on these findings, it was concluded that the increase in the size and the number of carotenoid granules and also the carotenoid content in the ventral skin were very important for red body coloration during the growth of this species (Matsui *et al*., 2003).

There are two ways for formation of the body coloration in animals. One is that body color is produced by auto-synthesis of pigments. The other takes place by the uptake of pigments directly from foods. Since animals are unable to synthesize carotenoids by themselves (Goodwin, 1986), they must take up pigments *via* foods for their body coloration. To understand the reason for this change in coloration of the ventral skin of the newt, it is necessary to investigate the food habit of this species. Here we report for the first time the food habits of young juvenile *C. pyrrhogaster* having the ventral creamy skin and other ones with the red skin. The food habit of *H. nebulosus* is also reported.

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MATERIALS AND METHODS

Animals

Juveniles of the Japanese newt *C. pyrrhogaster* were collected from November 2000 to May 2002 on the dike that was slightly elevated trail between adjoining paddy fields in Fukue Island in Nagasaki Prefecture, Japan. The juveniles were divided into 2 groups based on their snout-vent length (SVL; SVL>30.0mm and SVL<29.9mm). Prey animals were obtained separately from 62 individuals by the stomach flushing and fixed in 70% ethanol (Joly and Giacoma, 1992; Ihara, 1998). Since stomach items were enveloped in an opaque membrane, the membrane was removed by using fine-tipped forceps to identify the ingested species under a stereoscopic microscope (Aoki, 1999). The sample was then put on a piece of filter paper (Whatman No. 5) to eliminate an excess of 70% ethanol around the body. Total weight of prey animals in the same family was determined first using an electronic balance (Sartorius, model BP150), since each animal was too light to be weighed. Then, the average weight of each prey animal was calculated by dividing the total weight by the number of prey animals weighed. After the stomach flushing, the juveniles were released at the same location at which they had been captured.

Sampling of soil animals

Soil animals were obtained in May 2002 from the habitat of the juvenile newts and salamanders. The soil was scooped from 2 grids (W×L×D=25×25×5 cm). Then, animals were collected routinely by exposure of the soil to a 40-watt bulb for 48 hr using the Tullgren funnel.

Determination of the body color of newts

The body color of 21 juveniles collected in May 2002 was determined under daylight using the Color chart 1368 printed in the CMYK style (Optic design laboratory, Tokyo) (Sawada, 1963).

Statistical analysis

Kendall's rank correlation coefficient (Sokal and Rohlf, 1973) was used to test the significance of the food palatability and the chi-square (χ^2) test (Sokal and Rohlf, 1973) for the significance of the food compositions between the smaller and larger juveniles.

RESULT

We identified 402 prey items washed out from the stom-

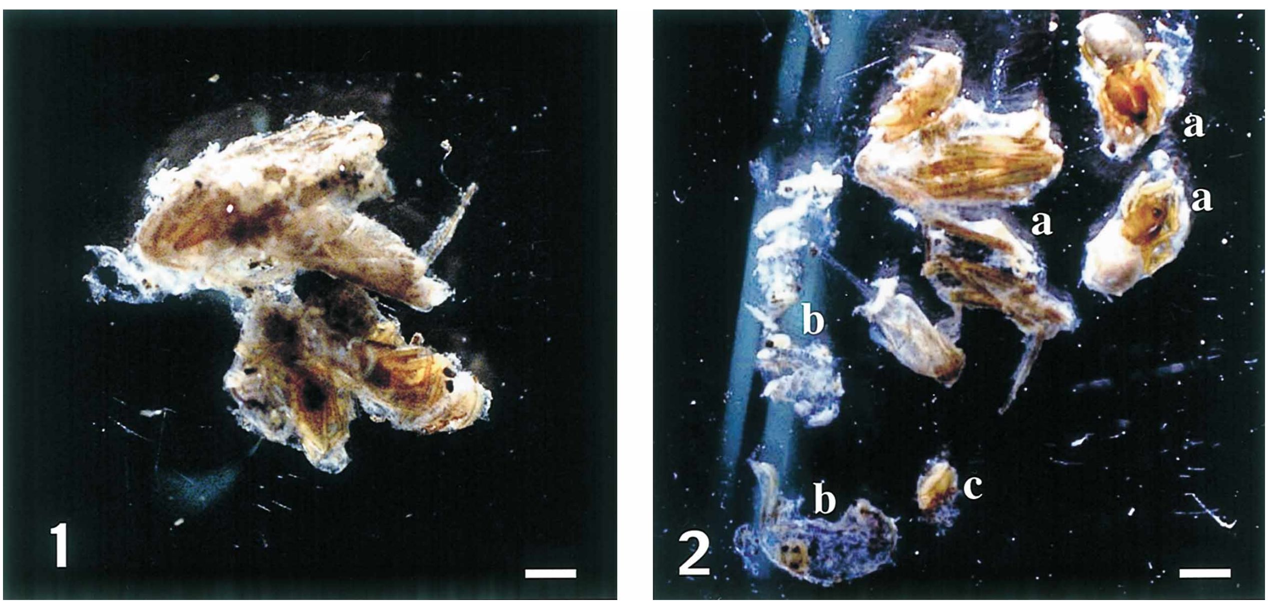


Fig. 1. Prey animals extracted from the stomach of a juvenile newt. Prey animals prior to (1) and after (2) removal of the envelope are shown in this figure. Each prey animal in the stomach was enveloped in the light brown membrane and the prey had coalesced into a mass. a, Araneae. b, Collembola. c, Acari. Bar=1mm.

Table 1. Number and size of *C. pyrrhogaster* and *H. nebulosus* collected.

	C. pyrrhogaster				*H. nebulosus*			
	Number of juveniles	Number of juveniles with diets	SVL ± SD (mm) (Range)	Mouth width ± SD (mm) (Range)	Number of individuals	Number of juveniles with diets	SVL ± SD (mm) (Range)	Mouth width ± SD (mm) (Range)
Autumn 2000	11	11	21.90±4.04 (19.0–33.2)	4.77±0.78 (4.10–6.80)	3	3	42.93±21.17 (22.79–65.0)	–
Spring 2001	16	15	24.61±5.58 (20.5–40.1)	5.65±1.35 (4.20–7.10)	–	–	–	–
Autumn 2001	14	12	30.1±6.27 (21.2–39.9)	6.22±0.82 (4.70–7.40)	6	5	47.40±13.09 (31.10–67.90)	7.73±1.20 (6.20–9.10)
Spring 2002	21	20	30.28±5.55 (23.4–40.8)	6.01±0.95 (4.80–7.80)	–	–	–	–
Total	62	58	27.34±6.40 (19.0–40.8)	5.65±0.42 (4.10–7.80)	9	8	45.91±14.97 (22.79–67.90)	7.73±1.20 (6.20–9.10)

Number of juveniles with or without stomach contents, SVL and mouth width are shown in this table.

Table 2. Relationship between the size and the color of the ventral skin of juvenile newts.

(A)

Color index					S	L
Magenda	Yellow	Cyan	Black		SVL< 29.9 mm	SVL> 30.0 mm
70	90	0	0		2	1
70	90	0	10		1	
70	100	0	10		2	
80	100	0	0		1	
80	90	0	10			1
80	100	0	10		5	3
90	90	0	10			2
100	100	0	20			2
100	100	0	30			1
				total	11	10

(B)

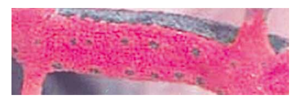
color index

70-90-0-0

small (SVL=21.2 mm)

80-100-0-10

medium (SVL=28.7 mm)



100-100-0-20

large (SVL=37.1 mm)

(A) Twenty-one juvenile newts were captured in May of the year 2002 on Fukue Island and the color of the ventral skin was determined as described in MATERIALS AND METHODS.
(B) Color index was given below the picture of the ventral skin of small, medium and large juveniles.

Table 3. Dietary compositions of *C. pyrrhogaster* and *H. nebulosus* collected from the same area in the paddy field in Fukue Island.

	C. pyrrhogaster Frequency of prey animals (Number %)										*H. nebulosus* (Number %)	Soil animals (Number %)
Season	Autumn 2000		Spring 2001		Autumn 2001		Spring 2002		total		Autumn 2000, 2001	Spring 2002
	S	L	S	L	S	L	S	L	S	L		
Number of juveniles	10	1	14	2	8	6	13	8	45	17	9	
Number of prey	23	14	44	25	54	42	107	93	228	174	23	203
Nematoda	–	–	4.5	–	–	–	–	–	0.9	–	–	
Gastropoda	8.7	–	2.3	–	2.0	4.3	7.5	5.4	5.4	4.6	–	–
Megascolecidae	–	–	–	–	–	–	–	–	–	–	4.4	0.5
Acari	17.4	7.1	22.7	12.0	24.0	12.9	29.0	9.7	25.4	12.6	4.4	62.1
Arcaneae	–	–	13.6	12.0	8.0	–	1.9	6.5	5.4	5.2	4.4	1.5
Armadillidae	–	–	6.8	–	2.0	–	–	–	0.4	–	–	–
Porcellionidae	–	–	–	–	–	–	0.9	–	1.8	–	–	–
Amphipoda	4.3	64.3	–	4.0	–	–	2.8	1.1	1.8	6.3	13	–
Doratodesmidae	–	–	–	–	–	–	–	–	–	–	56.5	–
Polyxenida	–	–	–	–	–	–	12.1	7.5	5.8	4.0	–	1.0
Myriapoda	4.3	–	–	–	–	1.4	–	–	0.4	1.1	4.4	6.4
Collembola	39.1	7.1	34.1	28.0	54.0	28.5	15.9	55.9	30.4	45.4	4.4	12.8
Thysanura	–	–	–	–	–	–	–	2.2	–	1.1	–	–
Aphidoidea	4.3	14.3	6.8	16.0	–	1.4	0.9	–	0.4	4.0	–	–
Enicocephalidae	–	–	–	–	–	–	0.9	1.1	0.4	0.6	–	–
Cicadidae	4.3	–	–	–	–	–	–	–	0.4	–	–	–
Thysanoptera (A)	–	–	–	–	2.0	–	–	–	0.4	–	–	–
Coleoptela (A)	8.7	7.1	–	–	2.0	1.4	19.6	2.2	10.3	2.8	4.4	4.9
Coleoptela (L)	4.3	–	–	–	–	1.4	3.7	2.2	2.7	1.1	–	–
Diptela (A)	–	–	–	4.0	–	–	–	–	–	0.6	–	–
Diptela (L)	–	–	9.1	20.0	–	8.6	1.9	4.3	2.7	8.6	4.4	–
Formicidae	4.3	–	–	4.0	6.0	–	2.8	1.1	3.1	1.1	–	10.8

	C. pyrrhogaster Wet weight (%)										*H. nebulosus* Wet weight (%)	Soil animals Wet weight (%)
Season	Autumn 2000		Spring 2001		Autumn 2001		Spring 2002		total		Autumn 2000, 2001	Spring 2002
	S	L	S	L	S	L	S	L	S	L		
Number of juveniles	10	1	14	2	8	6	13	8	45	17	9	
Number of prey	23	14	44	25	54	42	107	93	228	174	23	203
Nematoda	–	–	*	–	–	–	–	–	*	–	–	–
Gastropoda	11.4	–	4.3	–	10.2	7.9	9.1	8.6	11.7	5.4	–	–
Megascolecidae	–	–	–	–	–	–	–	–	–	–	90.5	69.30
Acari	4.2	0.7	7.9	2.1	22.5	4.3	6.4	2.8	10.1	2.7	0.1	10.40
Arcaneae	–	–	7.6	3.4	12.0	–	0.7	3.0	3.4	1.8	0.2	0.40
Armadillidae	–	–	*	–	*	–	–	–	*	–	–	–
Porcellionidae	–	–	–	–	–	–	*	–	*	–	–	–
Amphipoda	15.3	90.5	–	10.3	–	–	9.1	4.6	10.4	19.7	4.7	–
Doratodesmidae	–	–	–	–	–	–	–	–	–	–	0.9	–
Polyxenida	–	–	–	–	–	–	24.2	19.9	20.8	7.7	–	1.50
Myriapoda	12.2	–	–	–	–	5.6	–	–	–	1.4	1.3	12.50
Collembola	8.1	1.2	10.2	4.3	43.8	8.3	3.0	14.2	2.6	8.5	0.1	1.90
Thysanura	–	–	–	–	–	–	–	*	–	*	–	–
Aphidoidea	5.4	0.6	12.1	14.4	–	2.5	1.1	–	0.9	4.4	–	–
Enicocephalidae	–	–	–	–	–	–	*	*	*	*	–	–
Cicadidae	*	–	–	–	–	–	–	–	*	–	–	–
Thysanoptera (A)	–	–	–	–	*	–	–	–	*	–	–	–
Coleoptela (A)	3.6	7.0	–	–	3.2	0.8	7.5	–	6.5	0.8	0.2	1.40
Coleoptela (L)	38.3	–	–	–	–	17.6	30.4	23.1	26.1	13.5	–	–
Diptela (A)	–	–	–	*	–	–	–	–	–	*	–	–
Diptela (L)	–	–	57.9	64.5	–	53.0	7.6	23.2	6.6	33.8	2.0	–
Formicidae	1.5	–	–	1.0	8.2	–	0.9	0.5	0.8	0.4	–	2.70

(Four-hundred-two and 23 prey animals were obtained from 62 juvenile *C. pyrrhogaster* and 9 *H. nebulosus*, respectively, by stomach flushing. The asterisk(*) in this table indicates that the wet weight could not be determined because of weight limit (0.001g).

ach of 62 juvenile newts that were collected on Fukue Island in Nagasaki Prefecture. As shown in Fig. 1, prey animals in the stomach were trapped within an opaque envelope. However, there was no difficulty to identify them under a stereoscopic microscope after the envelope had been removed. Table 1 shows the number of newts and salamanders, which we collected for this study, and the SVL and the mouth width of them. Fifty-eight out of the 62 newts had the stomach contents (Table 1). Table 2 shows the relationship between the size (SVL) and the color of the ventral skin of the juvenile newts. The color of the ventral skin of the smaller juveniles (S) appeared creamy, but 9 out of 10 larger juveniles (L) had the ventral red skin. Table 3 shows food habits of the newt *C. pyrrhogaster* and the salamander *H. nebulosus*. In the smaller juveniles (SVL<29.9mm), Collembola and Acari, common in the soil, were the prey animals dominant in number, representing 30.4% and 25.4%, respectively of the ingested prey (Table 3). Statistical analysis of the animals extracted from the stomach and those living in the soil gave a Kendall's rank correlation coefficient of 0.451 (p=0.019), indicating a good correlation between the two. However, Collembola constituted only 7.9% and Acari 7.7% of the total weight of the stomach content extracted from the smaller juvenile newts (Table 3). In the larger juvenile newts (SVL>30.0mm) as well, Collembola (45.4%) and Acari (12.6%) were dominant, and the former constituted only 8.5%, and the latter, 2.7%, of the total weight of the stomach content as well (Table 3). No significant difference was observed in the diet compositions between the smaller and larger juveniles (the χ^2-test; χ^2=37.07, df=12, p=0.065). Furthermore, we could not find any seasonal changes in the food palatability either. The newts ate Collembola and Acari mainly in number in spring and autumn. As also seen in Table 3, Doratodesmidae (56.5%) was the most dominant prey animal of *H. nebulosus* in terms of number, but constituted only 0.9% of the total weight of the stomach content (Table 1). On the other hand, Megascolecidae (4.4%) was numerically a minor prey animal of *H. nebulosus*, but constituted 90.5% of the total weight of the stomach content. Neither Doratodesmidae nor Megascolecidae was found among prey items extracted from the stomach of *C. pyrrhogaster*.

DISCUSSION

In the present study, diet compositions of Japanese newts during the juvenile period were examined. The juvenile newt *C. pyrrhogaster* consumed Acari and Collembola as their main prey animals, whereas the salamander *H. nebulosus* preferred Doratodesmidae. Doratodesmidae was not observed in prey items extracted from the stomach of the juvenile newts. To date there has been only 1 report showing the food palatability of the juvenile and adult salamander *Hynobius tokyoensis*, a Japanese urodeles (Ihara, 1998). According to Ihara (1998), this salamander eats Porcellionidae and Trochelipidae. To the best of our knowledge, this is the first report describing the food habits of juvenile *C. pyrrhogaster* and *H. nebulosus*. Food habits of several species in the foreign urodela have been reported (Hamilton, 1932; Morgan and Grierson, 1932; Martof and Scott, 1957; Bury and Martin, 1973; Powders and Tietjen, 1974; Burton, 1976; Verrell, 1987; Wisniewski, 1989). Adults of 5 foreign species of urodeles have food palatability different from one other (Burton, 1976). Regarding the food habits of foreign juvenile salamanders, there is 1 report showing that the size of prey animals becomes bigger as juvenile salamanders grow, but that the juveniles do not change the food palatability (Maglia, 1996). In our study the smaller and larger juvenile newts ate Acari and Collembola dominantly as their prey animals. Thus, it is reasonable to conclude that *C. pyrrhogaster* does not change its food habit during the juvenile period. Since Acari and Collembola are very tiny and light, however, they constitute only a few percents of the total weight of the stomach content extracted from *C. pyrrhogaster*. Therefore, to keep active, juvenile newts need to eat other heavier soil animals such as Coleoptela, Gastropoda, Aphidoidae, Amphipoda and Diptela. In this regard, *H. nebulosus* eat Megascolecidae. Thus, the food habit of juvenile *C. pyrrhogaster* clearly differs from that of *H. nebulosus*, although juveniles of the 2 species inhabit in the same area.

As mentioned earlier, the color of the ventral skin of smaller juveniles is creamy, but that of larger juveniles is red (Matsui *et al*., 2003). The color of the ventral skin of the Japanese salamander *H. nebulosus* is different from that of the newt *C. pyrrhogaster*. To change the color of the ventral skin red, juvenile newts need to eat foods containing carotenoids, because they cannot synthesize carotenoids by themselves (Goodwin, 1986). The red coloration of the ventral skin of *C. pyrrhogaster* was closely related to the size and the number of carotenoid granules and the content of carotenoids in the granules (Matsui *et al*., 2003). As the juvenile newts do not change the food palatability during the red body coloration, the body coloration may be caused by the constant accumulation of carotenoids in the skin. However, we cannot exclude the possibility that older juvenile newts eat soil animals carrying a large amount of carotenoids, but they may have not been found in this study. To prove this, we must investigate which prey animals contain larger amounts of carotenoids, and what kinds of carotenoids are contained in the prey animals. Nevertheless, it is evident that the food habit of *C. pyrrhogaster* differs from that of *H. nebulosus*. The red body coloration of the newt *C. pyrrhogaster* is probably not due to a change in the food habit of the animal during the juvenile period.

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