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Authors: Ozaki, Kotaro, and Nishikawa, Kanto

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Feeding Habits of the Japanese Fire-belly Newt (Ampbihia: Urodela: Salamandridae) in Central Honshu, Japan

KOTARO OZAKI[1,*] AND KANTO NISHIKAWA[1,2]

[1]*Graduate School of Human and Environmental Studies, Kyoto University, Yoshida Nihonmatsu-cho, Sakyo, Kyoto 606–8501, JAPAN*
[2]*Graduate School of Global Environmental Studies, Kyoto University, Yoshida Honmachi, Sakyo, Kyoto 606–8501, JAPAN*

Abstract: The Japanese fire-belly newt, *Cynops pyrrhogaster*, is a near threatened species that is conservation dependent. Here, we examine feeding habits across the year within a genetically divergent intraspecific lineages of this species (the Central Lineage) to provide information to support future in-situ and ex-situ conservation activities. Stomach contents from newts were collected in a paddy field habitat, Kyoto City, central Honshu, Japan for two consecutive years. Throughout the year, dipteran aquatic larvae were the most important food source both for males and females, although terrestrial invertebrates were also important prey for the newts. During periods of low prey availability shed skin could also be a relatively valuable source of food. Sympatric frogs and newts appear to target different prey and this may facilitate their co-existence. Our study provides new information on suitable prey items for the Central Lineage of *C. pyrrhogaster* both in its natural habitat and in captivity.

Key words: Central Lineage; *Cynops pyrrhogaster*; Japan; Phenology; Prey item

INTRODUCTION

The Japanese fire-belly newt, *Cynops pyrrhogaster*, belongs to the subfamily Pleurodelinae, which occurs in whole Eurasia and North America, and the species widely inhabits Honshu, Shikoku, and Kyushu islands, Japan. It was a common species inhabiting wetlands, slow-flowing streams, rice paddies, and irrigation canals. However, recent habitat loss of amphibians due to anthropogenic activities and overhunting for pets have reduced their populations, like many other amphibians all over the world (Baillie et al., 2004; Cushman, 2006). The Japanese fire-belly newt is currently listed as Near Threatened (NT) in the Red Data Book of the Ministry of the Environment of Japan.

In order to conserve this widely distributed newt, we must pay attention for conserving its intraspecific geographic variation. The geographic variation of the newt has been studied in detail and at least four major groups have been delineated by morphological, behavioral, biochemical, and molecular phylogenetic studies (Sawada, 1963a, b; Hayashi and Matsui,

* Corresponding author.
E-mail address: imorigame@outlook.jp

1988, 1990; Tominaga et al., 2013). These groups may be separated into several taxa for conservation units in future.

The Central Lineage (sensu Tominaga et al. [2013]) of *C. pyrrhogaster* occurs in the Kansai District, where the habitat of the species has been greatly reduced, and it is listed in the local red lists (e.g., Kyoto Prefecture where this study was conducted [Kyoto Prefecture Department of the Environment, 2015]). In the future, some of the threatened populations of the Central Lineage will be protected and some will be maintained in specific protected area and/or in ex-situ conservation areas. In such case, basic ecological information of the conservation unit is likely to greatly improve the effectiveness of targeted conservation actions. However, ecological information for the Central Lineage is quite limited. There have been some studies on the feeding habits of the other lineages of *C. pyrrhogaster* although the numbers of individuals examined was small and no seasonal and sexual comparisons were made (Southern Lineage: Matsui et al. [2003]; Northern Lineage: Nakagawa and Kusano [2007], Ihara [2009, 2014]). These studies were done in a range of habitats, including artificial pond (Nakagawa and Kusano [2007]: seminatural artificial pond in a campus of the Tokyo Metropolitan University, Hachioji City, Tokyo), paddy fields (Matsui et al. [2003]: Fukue Island in Nagasaki Prefecture and Ihara [2009]: a mountain area in Fukushima Prefecture), and natural ponds (Ihara [2014]: Inawashiromachi, Fukushima Prefecture). The environment at the study site in our study is similar to that of Ihara (2009), in which the dipteran larvae were reported as main prey items.

In this study, we examined sexual and seasonal variations in the feeding habits of the Central Lineage of the Japanese fire-belly newt over two years, with the intent to contribute baseline information to underpin ex-situ conservation measure in future.

Materials and Methods

We caught *Cynops pyrrhogaster* once a week from 5 June 2020 to 15 November 2020 and 9 April to 21 September 2021 in a rice field at Iwakura, Kyoto, central Honshu, Japan (geographic coordinate not shown for conservation reasons). No newts were found from December 2020 to March 2021. The rice field is surrounded by an evergreen coniferous forest zone and artificially planted Japanese cedar forest (*Cryptomeria japonica*) at an elevation of about 150 m. Seven species of frogs, *Dryophytes japonicus*, *Fejervarya kawamurai*, *Glandirana rugosa*, *Pelophylax nigromaculatus*, *Rana japonica*, *Zhangixalus arboreus*, and *Z. schlegelii*, occur in the study site. Of these species, *Rana japonica*, *Zhangixalus arboreus*, and *Z. schlegelii* only appear in the study site during their breeding seasons.

We caught newts for three hours between 1100 h and 1400 h at each survey using dip nets. After collection, the stomach contents were immediately regurgitated by injecting water (ca. 5 ml) into the stomach with a syringe without anesthesia and preserved in 70% ethanol for subsequent analyses. For all newts captured we measured snout-vent length (SVL: length from the tip of snout to the anterior end of cloaca; to nearest 0.1 mm) and mouth width (MW; to nearest 0.1 mm), weighed body mass (BM; to nearest 0.1 g) and took picture of the ventral surface for individual identification. The sex of each newt was determined based on morphological features of the vent and tail (males have distinctly swollen vent lobes and breeding-color on the tail in the breeding season, females do not have swollen vents and have longer tails than males). Within the day of capture, we released newts where they were captured. Sexual differences in SVL, MW, and BM were determined by using t-test.

We identified stomach contents to the lowest practical taxonomic level, which was usually to class, order or family for all the prey groups. We measured maximum length and width of each prey item (excluding antennae and cerci) to the nearest 0.1 mm using either a caliper or a calibrated ocular micrometer attached to a dissecting microscope. For partially digested prey items, we estimated lengths (L) based on their widths (W) using predetermined length-width regressions from intact putative prey organisms that we sampled in the field. Volumes of prey items were approximated using the formula for an ellipsoid (Dunham, 1983):

$$V=4/3\pi(L/2)(W/2)^2.$$

We gathered other stomachs including shed skin and inorganic substances, mainly pebbles, tightly into hemispheres and measured their diameters. Their volumes were calculated using the formula for a hemisphere:

$$V=2/3\pi r^3.$$

We estimated relationships between predator and prey sizes by regression of volumes of the largest and smallest prey items in a stomach on the newt SVL and by calculating correlation coefficients. Only newts with at least three prey items in their stomachs were included in this analysis.

The examined variation by month in the average number of food items per newt and the vacuity index (=empty stomach individuals [n]/total individuals [n]×100).

The importance of each prey taxon was calculated by the index of relative importance (IRI) of Pinkas (1971) and its percentage ratio as follows:

IRI=(Volumetric proportion [%]+Numeric proportion [%])×Frequency (%).

pIRI=(IRI of a taxon/total IRI)×100

We calculated the frequency of occurrence (=the numbers of newts which eat the prey/total number of stomach contents present and absent newts), the numeric proportion (=the prey number/total number of prey), and volumetric proportion (=total volume of taxon/total volume of prey).

Sexual difference in proportion of habitat of prey organisms (aquatic or terrestrial) was examined by Fisher's exact test.

Results

Individual variation in newts

We collected and identified a total of 322 newts, all captured in the water (no adult newts collected on the land), of which 66 were recaptured more than once (10 times at maximum and 2.30 times on average). There was no significant difference in number of recaptures between males (x±SE=2.28±0.51, n=60) and females (2.31±0.43, 77) (Mann-Whitney U-test, z=0.0798, P=0.936).

There were significant sexual differences in SVL, MW, and BM (Table 1). All measurements and weight showed significantly larger values for females than males when using the t-test (SVL: t=10.1, P<0.001; MV: t=5.56, P<0.001; BM: t=8.07, P<0.001).

Diet composition

A total of 971 prey items were extracted from the stomachs of 258 of the 322 newts (including recaptured individuals; prey number/stomach=2.92±0.72 [x±SE]). The remaining 64 newts captured had no prey items.

Except for plant materials and inorganic substance, frequency, numeric proportion, and volumetric proportion were calculated (Table 2). Sexual difference in prey habitat (aquatic or terrestrial) was not significant both in numeric and volumetric values. Terrestrial organisms, such as lepidopteran larvae, Oligochaeta, and Coleoptera, were frequent stomach contents (17.6% in pIRI).

The highest value of total pIRI was obtained in dipteran aquatic larvae (38.8%) followed by shed skin (13.0%), Isopoda (12.4%), aquatic Gastropoda (10.9%), and Oligochaeta (8.5%) in this order. Sexual differences in frequency of consumption were found in Isopoda (20.7% in male and 6.9% in female) and aquatic Gastropoda (4.0% in male and 17.1% in female).

Three invertebrate phyla (Arthropoda, Mollusca, and Annelida) predominated in the prey

TABLE 1. Snout–vent length (SVL, in mm) and mouth width (MW, in mm), and body mass (BM, in g) of the newt.

Number of the newts with stomach contents		Female	Male
		183	143
SVL (mm)	Minimum-Maximum	37.4–58.9	33.0–57.2
	Mean±SD	50.3±0.6	46.0±0.6
Mouth width (mm)	Minimum-Maximum	7.2–12.7	6.2–12.0
	Mean±SD	10.9±0.1	10.3±0.2
Number of the newts with stomach contents		Female	Male
		180	128
Body mass (g)	Minimum-Maximum	2.4–9.2	2.1–7.2
	Mean±SD	5.92±1.9	4.88±1.6

items (86.2% in pIRI). Arthropoda consumed included all four classes (Insecta, Arachnida, Crustacea, and Myriapoda) and constituted 66.8% in pIRI. Insecta included 11 orders and constituted 53.2% in pIRI.

Besides invertebrates, 14 newt eggs, 11 frog eggs (28 June and 13 July 2021) and two tadpoles (13 July 2021) were found in stomachs. Thirteen newt eggs were consumed by nine females and the remaining one egg was consumed by one male. The frog eggs were guessed to be the Wrinkled frog (*Glandirana rugosa*) because no other frogs spawned at the timing of its discovery. The tadpoles had already been digested, but these were possibly those of the Black-spotted pond frog (*Pelophylax nigromaculatus*) because only the tadpoles of this species were found in the canal at the time of survey.

Plant materials (plant bodies and seeds) and inorganic substance (pebbles and dirt) occupied 15.4% and 5.8% of the volumes of newt stomach contents, respectively.

There were no significant correlations between newt SVL and volumes of the largest and smallest prey taken (Fig. 1).

Seasonal variation

In seasonal variation, we focused on the five highest total pIRI prey because the prey items below the fifth rank were much less consumed (less than 5%) (Fig. 2-4). Phenological trends in pIRI were stable in Isopoda and Oligochaeta, but showed a gradual decrease in Diptera larvae (Table 2, Fig. 2). A seasonal peak was found in aquatic Gastropoda (July and August in 2020) and shed skin (September, October, and November in 2021), but only in one of the two consecutive years. Less-valued prey or non-prey contents showed a gradual decrease in frequency from August to November.

Sexual variation

Frequency of occurrence of all prey taxa did not differ significantly between the sexes except for stoneflies (Plecoptera) (Fisher's exact probability test, $P>0.05$ for 26 pray taxa. Bonferroni correction, stoneflies' P value= $0.0013<0.05/27$). In June 2020, males ate many more stoneflies than females, which caused significant sexual difference. The reason why the male ate such larger number of stoneflies in the month was unknown. Excluding June 2020 data, frequency of occurrence of stoneflies did not differ significantly between the sexes.

Highest pIRI was found in dipteran larvae in both sexes, but the second (Isopoda in males but aquatic Gastropoda in females) and fifth (Plecoptera in males but Isopoda in females) highest pIRI differed between sexes. Males showed higher pIRI than females in Diptera

TABLE 2. Diet composition of *Cynops pyrrhogaster* collected in 2020 and 2021 (971 prey from 322 newts, total volume 15,075.9 mm^3).

Prey taxa	Frequency (%)			Numeric proportion (%)			Volumetric proportion (%)			IRI			pIRI (%)		
	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female
Insecta															
Diptera larva	23.55	24.48	22.83	21.47	25.50	19.24	14.38	11.07	17.13	844.17	895.06	830.10	38.76	35.27	40.19
Diptera pupa	7.95	7.69	8.15	3.68	5.44	2.70	1.94	1.55	2.27	44.72	53.83	40.52	2.05	2.12	1.96
Ephemeroptera	2.14	1.40	2.72	6.95	0.57	10.49	1.19	0.02	2.16	17.43	0.83	34.39	0.80	0.03	1.66
Plecoptera	6.73	11.89	2.72	5.42	9.74	3.02	2.46	3.10	1.93	53.02	152.69	13.45	2.43	6.02	0.65
Hemiptera	3.98	4.90	3.26	3.78	1.72	4.93	0.83	0.69	0.95	18.35	11.78	19.18	0.84	0.46	0.93
Lepidoptera*	7.34	5.59	8.70	1.94	2.29	1.75	10.52	7.03	13.42	91.46	52.17	131.88	4.20	2.06	6.38
Hymenoptera*	5.50	5.59	5.43	2.04	2.29	1.91	3.40	3.12	3.63	29.95	30.27	30.08	1.38	1.19	1.46
Coleoptera*	3.36	1.40	4.89	0.92	0.29	1.27	1.03	0.54	1.44	6.57	1.16	13.27	0.30	0.05	0.64
Trichoptera	1.83	1.40	2.17	0.82	1.43	0.48	1.58	0.70	2.30	4.39	2.98	6.04	0.20	0.12	0.29
Odonata	1.22	1.40	1.09	0.41	0.86	0.16	3.39	1.51	4.96	4.65	3.31	5.56	0.21	0.13	0.27
Orthoptera*	5.81	8.39	3.80	2.15	4.01	1.11	5.39	8.16	3.09	43.81	102.16	15.98	2.01	4.03	0.77
Collembola*	0.61	0.70	0.54	0.41	0.86	0.16	0.01	0.01	0.00	0.25	0.61	0.09	0.01	0.02	0.00
Arachnida															
Araneae*	4.28	4.20	4.35	1.23	1.72	0.95	0.80	0.36	1.17	8.68	8.73	9.21	0.40	0.34	0.45
Acari*	0.31	0.70	0.00	0.20	0.57	0.00	0.00	0.00	0.00	0.06	0.40	0.00	0.00	0.02	0.00
Crustacea															
Isopoda	12.84	16.78	9.78	13.09	20.06	9.22	8.01	11.30	5.28	270.99	526.20	141.84	12.44	20.73	6.87
Myriapoda															
Diplopoda*	4.28	5.59	3.26	1.64	2.58	1.11	2.14	2.89	1.51	16.17	30.61	8.57	0.74	1.21	0.41
Annelida															
Oligochaeta*	7.34	6.99	7.61	2.56	3.15	2.23	22.72	24.47	21.27	185.52	193.15	178.75	8.52	7.61	8.65
Amphibia															
Anuran egg	1.53	2.10	1.09	1.12	1.43	0.95	0.54	0.79	0.33	2.54	4.67	1.39	0.12	0.18	0.07
Anuran larvae	0.61	0.70	0.54	1.43	0.29	2.07	0.61	0.53	0.67	1.25	0.57	1.49	0.06	0.02	0.07
Urodelan egg	3.06	0.70	4.89	0.20	0.29	0.16	1.00	0.36	1.53	3.69	0.45	8.27	0.17	0.02	0.40
Urodelan shed skin	14.98	13.99	15.76	5.01	6.30	4.29	13.89	19.69	9.07	283.25	363.53	210.64	13.01	14.32	10.20
Crustacean															
Potamidea	0.92	0.00	1.63	0.31	0.00	0.48	0.63	0.00	1.16	0.86	0.00	2.66	0.04	0.00	0.13
Podocopa	0.61	0.00	1.09	0.41	0.00	0.64	0.01	0.00	0.02	0.26	0.00	0.71	0.01	0.00	0.03
Anostraca	0.92	0.00	1.63	0.82	0.00	1.27	0.18	0.00	0.33	0.91	0.00	2.61	0.04	0.00	0.13
Diplostrace	1.22	2.10	0.54	6.75	0.00	10.49	0.06	0.00	0.11	8.33	0.00	5.76	0.38	0.00	0.28
Mollusca															
Aquatic Gastropoda	12.84	9.79	15.22	15.13	8.31	18.92	3.28	2.08	4.28	236.56	101.75	353.08	10.86	4.01	17.09
Terrestrial Gastropoda*	1.53	3.50	0.00	0.10	0.29	0.00	0.00	0.01	0.00	0.16	1.03	0.00	0.01	0.04	0.00
Aquatic organisms	—	—	—	83.32	81.14	84.54	63.75	62.84	64.52	—	—	—	82.43	83.44	81.22
Terrestrial organisms	—	—	—	16.68	18.86	15.46	36.25	37.16	35.48	—	—	—	17.57	16.56	18.78

*: Terrestrial organisms

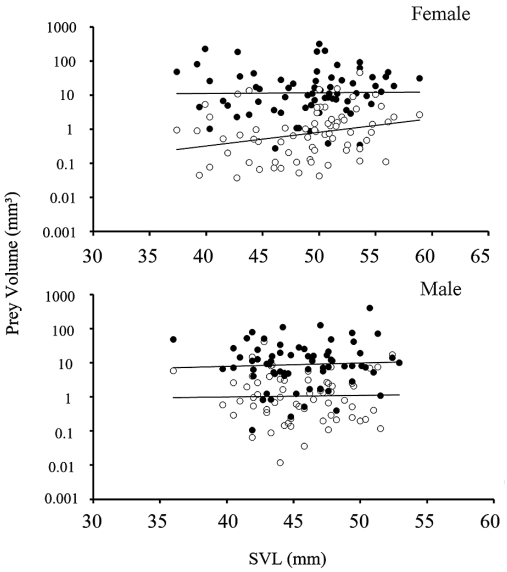

FIG. 1. Relationship between newt SVL in minimum (open circles) and in maximum (closed circles) volumes of prey in stomach content. (Female: n=70, rmin=0.265, rmax=0.017; Male: n=68, rmin=0.022, rmax=0.052).

larvae in August 2020, shed skin in September 2021, and aquatic Gastropoda in July 2020, but females did in Isopoda in August 2020. No sexual difference was consistently found across the two years, suggesting annual variation is greater than sexual variation. Although the pIRI was small (0.40% in females and 0.02% in males), females consumed much more urodelan eggs (conspecific ones) than males (Table 2).

DISCUSSION

In our study site arthropods were the major prey item of the Japanese fire-belly newt, and dipteran larvae were most important prey irrespective of year and sex at all times except for winter. Dipteran larvae (lake flies and soldier flies) were also the main food source of the newt population inhabiting a semi-natural artificial pond in the Kanto District (Nakagawa and Kusano, 2007), where newt populations belong to the Northern lineage sensu Tominaga

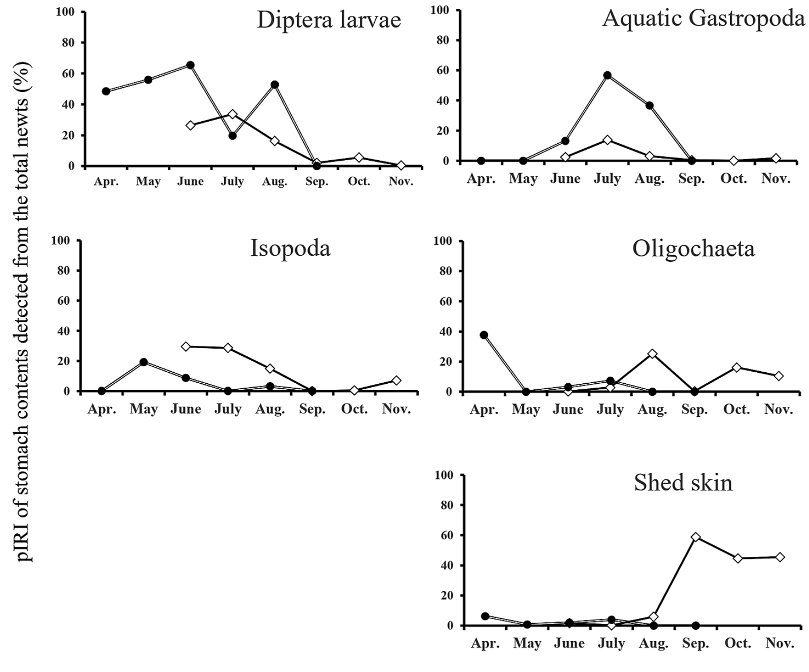

FIG. 2. Seasonal variation in pIRI of individual stomach contents of total *Cynops pyrrhogaster* in 2020 (closed circles) and 2021 (open diamonds).

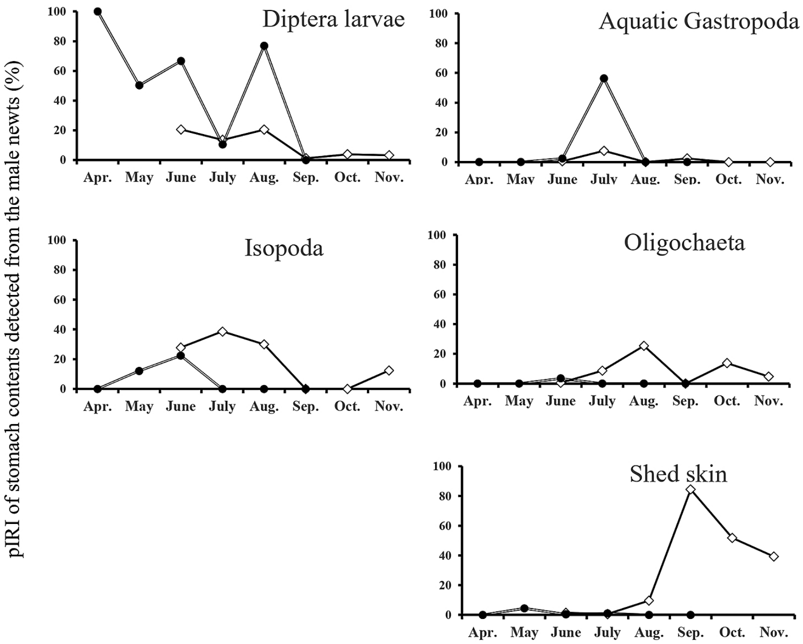

FIG. 3. Seasonal variation in pIRI of individual stomach contents of male *Cynops pyrrhogaster* in 2020 (closed circles) and 2021 (open diamonds).

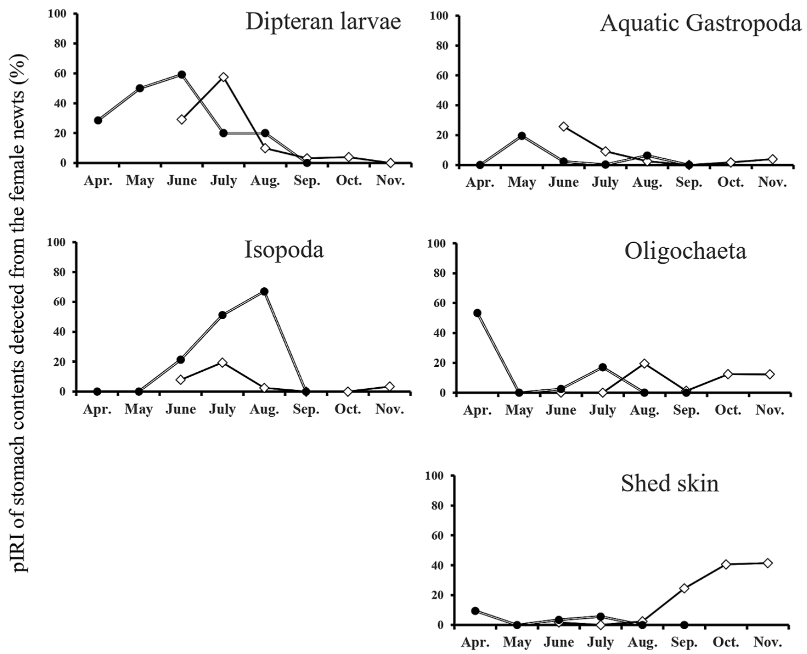

FIG. 4. Seasonal variation in pIRI of individual stomach contents of female *Cynops pyrrhogaster* in 2020 (closed circles) and 2021 (open diamonds).

et al. (2013). In addition, Diptera are the main prey item in a paddy field in Ishimushiro, Fukushima (Ihara, 2009) and in the sub-alpine Yachidaira high moor, Fukushima (Ihara, 2014). Therefore, it seems likely that aquatic larvae of Diptera are an important food source for newts irrespective of their lineages. This stands in contrast to the rate of consumption of terrestrial and active arthropods (ants, beetles, adult flies, caterpillars, and spiders) by sympatric frogs from Kyoto City and adjacent areas (see Hirai and Matsui, 1999, 2000, 2001).

This difference may be caused by prey-detection and predation methods of these animals. Terrestrial frogs detect their prey by eyes and catch it by tongue prehension or biting, thus they cannot catch tiny (and also immobile) prey. From our field observations, the newts spent much time for smelling the mud; it is considered that olfactory detection of prey is a key hunting strategy, as observed in other newt species (Copeland, 1913). Prey detection via olfaction in the newts could make enable sympatric cooccurence with frogs through increased food source segregation (Copeland, 1913).

The importance of the main prey of dipteran aquatic larvae decreased in autumn, while the importance of shed skin increased (but only in 2021). It is unlikely that the skin could compensate for the decreased prey in autumn, however the consumption of the skin in autumn may have some biological meaning, which will be a future challenge to explore.

In our study, terrestrial organisms accounted for only 16.7% of the total numeric proportion, but 36.3% of the volumetric proportion of food consumed. This may suggest that terrestrial invertebrates, like Oligochaeta (earthworm), enter the water accidentally and greatly contribute to the diet in volumetric proportion of this aquatic newt, or that some terrestrial invertebrates might be also preyed on outside of the water. In order to explore the newt feeding ecology throughout its whole life, we must survey not only aquatic phase but also the terrestrial phase of the newt.

Although we detected some sexual difference in prey contents, the annual differences were more marked. The annual difference in importance of the prey may be caused by annual fluctuation of prey biomass. Unfortunately, we did not examine prey biomass data in the field and cannot test this hypothesis with the data available. However, the newt is probably an opportunistic feeder (Ihara, 2014) and opportunistically feeds on the common available prey at the time. In order to protect the natural populations of the Central Lineage of the newt, it is necessary that terrestrial and aquatic environments, that produce a variety of prey fauna to compensate for biomass fluctuations of each prey. For conservation in captive condition, stable feeding of dipteran larvae like bloodworm and increasing the diversity of prey animals including terrestrial ones will be important for keeping the newts in a natural condition.

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