Current Herpetology 20(2): 97–103, December 2001
© 2001 by The Herpetological Society of Japan

Diet Composition of the Indian Rice Frog, *Rana limnocharis*, in Rice Fields of Central Japan

TOSHIAKI HIRAI AND MASAFUMI MATSUI*

Graduate School of Human and Environmental Studies, Kyoto University, Sakyo, Kyoto 606-8501, JAPAN

Abstract: *Rana limnocharis* from rice fields of Nara, central Japan, fed on a wide variety of prey animals. Arthropods constituted 94.8% in number and 52.9% in volume of total prey items. Among prey taxa, ants (26.9%) comprised the largest proportion in number, and earthworms (36.2%) in volume. One froglet and one tadpole were also recovered from frog stomachs. The generalized food habits of *R. limnocharis* would ensure the establishment of populations of this species outside its natural range.

Key words: *Rana limnocharis*; Food habits; Insectivore; Artificial introduction; Colonization

INTRODUCTION

Rana limnocharis is one of the most broadly distributed species of Asian anurans, ranging from East Asia through Southeast Asia to India (Maeda and Matsui, 1999). In Japan, this species is distributed in the central Ryukyus, Kyushu, Shikoku, and an area from the Chugoku District to the Tokai District of Honshu (Maeda and Matsui, 1999). Other than one exotic species, *R. catesbeiana*, *R. limnocharis* is the only amphibian species distributed in both the Ryukyu Archipelago and the mainislands of Japan.

Rana limnocharis is considered to have been artificially introduced into various parts of the Kanto District during the last decade (Osawa, 1998; Hasegawa and Ogano, 1998; Hayashi et al., 2000; see Hayashi and Kimura [2001] for review). The introduced *R. limnocharis* may have an unfavorable influence on indigenous anuran populations of each locality. However, *R. limnocharis* from Japan has never been studied from a viewpoint of community ecology: currently available information relevant to the natural history of this frog only concerns its embryonic temperature tolerances (Kuramoto, 1967), growth (Shichi et al., 1988), and vocalizations (Hata and Nagoshi, 1995). The paucity of studies concerning ecological relationships of coexisting anuran species makes it difficult for us to predict the effects of introduced *R. limnocharis* on the native anuran populations.

Food is an important niche axis for partitioning coexisting anurans (Toft, 1985), and food niche overlap (or diet similarity) has been used to hypothesize potential

* Corresponding author. Tel: +81-75-753-6846; Fax: +81-75-753-6846.

E-mail address: fumi@zoo.zool.kyoto-u.ac.jp (M. Matsui)

interactions between coexisting species (Inger and Greenberg, 1966; Stewart and Sandison, 1972; Miller, 1978; Licht, 1986; McAlpine and Dilworth, 1989; Werner et al., 1995). In the mainislands of Japan, food habits have been studied for *R. nigromaculata*, *R. porosa brevipoda*, *R. rugosa*, and *Hyla japonica* inhabiting rice fields (Hirai and Matsui, 1999; 2000a, b, 2001a, b). With respect to food habits of Japanese populations of *R. limnocharis*, however, a list of prey items recovered from four individuals (Tomida, 1976) is the only available information to the present.

In this paper, we present diet composition of *R. limnocharis* inhabiting rice fields of central Japan, and compare it with published data for conspecific populations from outside of Japan.

METHODS

We collected frogs from rice paddy fields in Nara City, Nara Prefecture, central Japan (34°42'N, 135°51'E). Collections were conducted at night between 2100 h and 2300 h on 20 June, 14 July, and 6 August 1997. Immediately after we captured frogs, we extracted their stomach contents by forced regurgitation with forceps, and preserved the contents in 10% buffered formalin for later identification and analysis. For each frog, we measured snout-vent length (SVL), mouth width and body mass, and examined the presence or absence of male secondary sexual characters (nuptial pads, vocal sacs, and an M-shaped dark marking on the throat). Individuals were divided into three sex-age classes: juvenile, adult male, and adult female. Adult males are easily differentiated from the others in possessing the male secondary sexual characters, but adult females and juveniles, both lacking those characters, are not distinguishable from each other with certainty unless the gonads are inspected by dissection. Nevertheless, because females are known to reach sexual maturity at larger body size than males (Maeda and Matsui, 1999), for we practical purposes, we regarded individuals smaller than the smallest adult male (actually 32.2 mm in SVL) as juveniles, and larger individuals as adult females, including sub-adults. In addition, we clipped toes in unique combinations for individual identification. After these procedures, we released frogs where they were captured.

In the laboratory, we identified stomach contents to the lowest practical taxonomic level, and measured maximum length (L) and width (W) of each prey item to the nearest 0.1 mm using either a caliper or a calibrated ocular micrometer fitted to a dissecting microscope. For partially digested prey items, we estimated lengths by measuring width and then using predetermined length-width regressions from intact prey (see Hirai and Matsui [2001c] for more details). Volume (V) of each prey item was calculated using the formula for an ellipsoid:

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

RESULTS

Diet composition

Forty frogs (17 adult females, 15 adult males, eight juveniles) were captured, of which six (five adult females, one adult male) had empty stomachs. The bulk of the diet was represented by three invertebrate phyla (Arthropoda, Mollusca, and Annelida), with Arthropoda including four classes (Insecta, Araneae, Crustacea, and Diplopoda), making up 94.8% in number and 52.9% in volume of total prey items (Table 1). Insecta contained seven orders, and comprised 62.3% in number and 31.0% in volume. Ants (Formicidae) comprised the largest proportion in number (26.9%), followed by spiders (Araneae; 20.3%) and dipterans (18.4%). In volume, however, earthworms (Oligochaeta; 36.2%) predominated in the

TABLE 1. Diet composition of *Rana limnocharis*, sampled from juveniles (J: 80 prey from eight frogs, total volume 332.18 mm^3), adult males (M: 69 prey from 14 frogs, total volume 1874.95 mm^3), and adult females (F: 63 prey from 12 frogs, total volume 1991.85 mm^3).

	Frequency of occurrence (%)				Numeric proportion (%)				Volumetric proportion (%)			
Prey taxa	J	M	F	Total	J	M	F	Total	J	M	F	Total
Arthropoda												
Insecta												
Hymenoptera												
Formicidae	87.5	35.7	33.3	47.1	50.0	14.5	11.1	26.9	7.9	0.7	1.0	1.4
Non-Formicidae	12.5	0.0	0.0	2.9	1.3	0.0	0.0	0.5	0.8	0.0	0.0	0.1
Coleoptera	12.5	0.0	33.3	14.7	1.3	0.0	7.9	2.8	0.1	0.0	21.0	10.0
Larvae	12.5	7.1	0.0	5.9	1.3	1.5	0.0	0.9	0.3	0.5	0.0	0.2
Diptera	50.0	35.7	50.0	38.2	5.0	31.9	20.6	18.4	0.6	1.8	0.9	1.3
Larvae	12.5	7.1	8.3	8.8	1.3	1.5	3.2	1.9	0.3	0.2	0.2	0.2
Lepidoptera	0.0	14.3	0.0	5.9	0.0	2.9	0.0	0.9	0.0	1.8	0.0	0.8
Larvae	0.0	0.0	16.7	5.9	0.0	0.0	3.2	0.9	0.0	0.0	1.9	0.9
Hemiptera	25.0	28.6	8.3	20.6	2.5	7.3	1.6	3.8	1.5	7.7	1.1	4.1
Dermaptera	12.5	0.0	0.0	2.9	1.3	0.0	0.0	0.5	21.6	0.0	0.0	1.7
Orthoptera	12.5	21.4	25.0	20.6	2.5	4.4	7.9	4.7	14.2	1.8	17.7	10.3
Arachnida												
Araneae	50.0	57.1	66.7	58.8	13.8	23.2	25.4	20.3	10.8	9.8	3.0	6.6
Crustacea												
Isopoda	37.5	21.4	25.0	26.5	8.8	4.4	9.5	7.6	32.9	11.2	14.9	14.6
Amphipoda	0.0	7.1	0.0	2.9	0.0	1.5	0.0	0.5	0.0	0.7	0.0	0.3
Diplopoda	25.0	7.1	8.3	11.8	8.8	1.5	1.6	4.3	3.1	0.1	0.2	0.4
Mollusca												
Gastropoda	12.5	14.3	16.7	17.7	2.5	2.9	3.2	2.8	6.0	0.2	3.1	2.0
Annelida												
Oligochaeta	0.0	7.1	16.7	8.8	0.0	1.5	3.2	1.4	0.0	52.6	25.7	36.2
Vertebrate												
Amphibia												
Anura	0.0	0.0	8.3	2.9	0.0	0.0	1.6	0.5	0.0	0.0	8.5	4.1
Larvae	0.0	7.1	0.0	2.9	0.0	1.5	0.0	0.5	0.0	11.0	0.0	4.9
Plant materials	37.5	50.0	33.3	41.2	—	—	—	—	—	—	—	—
Minerals	12.5	16.7	0.0	8.8	—	—	—	—	—	—	—	—

diet, followed by woodlice (Isopoda; 14.6%) and orthopterans (10.3%), and ants comprised a small proportion (1.4%).

Besides invertebrates, a recently metamorphosed froglet of *Hyla japonica* and an unidentified tadpole were recovered from the stomachs of medium-sized individuals (41.2 mm, and 37.2 mm in SVL, respectively). Plant materials and minerals also occurred in 41.2%, and 8.8%, respectively, of the stomachs examined. When food items were classified by their habitats, aquatic organisms (dipteran larvae, gerrid water-striders, amphipods, gastropods exclusive of land-snails and slugs, and anuran larvae) occurred in 32.4% of frog stomachs, but comprised only 6.1% and 9.3% in number and volume, respectively.

Comparisons among sex-age classes

Adult females, significantly larger than adult males in all measurements (SVL, mouth width, and body mass: Mann-Whitney U-test, $p<0.01$ for all, see Table 2), tended to take larger prey on average than did adult males and juveniles. Even so, significant difference was detected only between adult females and juveniles (Dunn's multiple comparison test, $p<0.05$). Although diet composition varied among sex-age classes (Table 1), only the frequency of ants showed statistically significant difference in the between-class comparisons: it was significantly higher in juveniles (87.5%) than in adult males (35.7%) and adult females (33.3%) (χ^2-contingency table test, df=2, $\chi^2=6.88$, $p<0.05$). Absence of significant differences in the number and volume of prey items among the three classes (Dunn's multiple comparison test, $p>0.05$), may be attributable to the within-class large variation, and/or small sample size for each class.

DISCUSSION

This study revealed that *R. limnocharis* from central Japan feeds predominantly on arthropods like most other ranid frogs hitherto studied (e.g., Jenssen and Klimstra, 1966; Houston, 1973; Whitaker et al., 1983; Hirai and Matsui, 1999). According to the past studies on the populations of *R. limnocharis* outside of Japan, arthropods comprised 89–100% in number of total prey items in the diet (Liu and Chen, 1933; Berry, 1965; Monhanthy-Hejmadi and Acharya, 1982). The value obtained by our study (94.8%) falls within this range.

Among diverse arthropod prey taxa, ants were reported to comprise the largest proportion in rice fields of China (27%; Liu and Chen, 1933), and in the swamps of Singapore (28%; Berry, 1965). These reported proportions are quite similar to the value in our result (26.9%). The previous studies, however, did not examine diet variations between juvenile and adult *R. limnocharis*. As shown in this study, the numeric proportion of ants is much larger in juveniles than in adults. This

TABLE 2. Means (±SD) of character dimensions of frogs and prey. Sample size and range are given in parentheses.

	Juveniles	Adult males	Adult females
Frogs			
SVL (mm)	29.3±2.7	35.8±2.9	41.4±3.5
	(8: 24.4–31.2)	(15: 32.2–40.2)	(17: 34.8–46.5)
Mouth width (mm)	10.1±0.9	11.9±1.0	13.6±1.3
	(8: 8.6–10.9)	(15: 10.9–14.0)	(17: 11.4–16.0)
Body mass (g)	1.9±0.4	3.6±0.9	6.2±1.6
	(8: 1.1–2.3)	(15: 2.4–5.6)	(17: 3.6–9.7)
Prey			
Length (mm)	3.6±0.9	5.0±1.5	7.6±4.9
	(7: 2.5–4.9)	(9: 3.3–8.3)	(9: 3.4–19.4)
Number/stomach	10.0±8.6	4.9±4.8	5.3±4.0
	(8: 2–30)	(14: 1–20)	(12: 1–13)
Volume/stomach (mm^3)	40.3±48.6	133.9±257.1	166.0±137.1
	(8: 0.7–149.0)	(14: 3.4–985.6)	(12: 5.4–384.7)

indicates that when diet composition is compared among populations, diet variation within a population should be considered.

Nevertheless, even if this kind of variation is considered, diet composition may differ among localities. For example, beetles (41%) were the most numerically predominant prey in India (Monhanthy-Hejmadi and Acharya, 1982) unlike in the other three localities. The second most important prey was hemipterans (16%) in China (Liu and Chen, 1933), termites (18%) in Singapore (Berry, 1965), ants (13%) in India (Monhanthy-Hejmadi and Acharya, 1982), and spiders (20.3%) in this study site. These local variations strongly suggest that the food habits of *R. limnocharis* are generalized, and that the frog changes diet menus flexibly in response to local variation in frequency of available prey items.

It is noteworthy that *R. limnocharis* consumed a tadpole and a froglet in this study site. Much larger frogs like the bullfrog (*R. catesbeiana*) often eat small vertebrates (see Bury and Whelan [1984] for review), but there are few reports that relatively small frogs like *R. limnocharis* feed on vertebrate prey. Even in *R. nigromaculata* and *R. porosa brevipoda*, only large individuals (SVL>53 mm) eat syntopic froglets (Hirai and Matsui, 1999; 2001a). Therefore, it may be safe to state that the food habits of *R. limnocharis* are characterized by diverse diet composition from tiny insects to small vertebrates. Such food habits seem to contribute at least partially to the success of this frog in establishing feral populations through artificial introduction (Osawa, 1998; Hasegawa and Ogano, 1998; Hayashi et al., 2000), because the generalized food habits of *R. limnocharis* should be advantageous in dealing with unfamiliar environments where diversity and abundance of prey may greatly differ from the original habitat.

The recently introduced populations of *R. limnocharis* in the Kanto District have received a great deal of attention (Osawa, 1998; Hasegawa and Ogano, 1998; Hayashi et al., 2000), because its colonization may lead to unfavorable influence on the native anuran populations. Further research is urgently needed to evaluate such influence and to determine whether or not to exterminate the introduced *R. limnocharis* for conservation of the native anurans. Since the colonized areas are still limited (Osawa, 1998; Hayashi and Kimura, 2001), extermination would be still possible.

Acknowledgments

We thank T. Hayashi and S. Osawa for providing literature.

Literature Cited

Berry, P. Y. 1965. The diet of some Singapore Anura (Amphibia). Proc. Zool. Soc. Lond. 144: 163–174.

Bury, R. B. and J. A. Whelan. 1984. Ecology and management of the Bullfrog. U. S. Dept. Int., Fish Wildl. Serv., Resour. Publ. 155: 1–23.

Hasegawa, M. and D. Ogano. 1998. Discovery of *Rana limnocharis* in the Boso peninsula: its distribution and status. (abstract) Jpn. J. Herpetol. 17: 193–194. (in Japanese)

Hata, N. and M. Nagoshi. 1995. Call analysis of two pond frogs, *Rana rugosa* and *R. limnocharis*. Bio. Inl. Wat. 10: 26–36. (in Japanese with English abstract)

Hayashi, T., K. Akaba, R. Ishizuka, and Y. Kimura. 2000. A record of the Indian rice frog, *Rana limnocharis*, from Tochigi Prefecture, central Japan. Bull. Tochigi Pref. Mus. 17: 109–112. (in Japanese with English abstract)

Hayashi, T. and Y. Kimura. 2001. *Rana limnocharis*. p. 90–91. In: Amphibians and Reptiles of Tochigi Prefecture. The natural environment section, the forestry department of Tochigi Prefecture, Utsunomiya. (in Japanese)

HIRAI, T. AND M. MATSUI. 1999. Feeding habits of the pond frog, *Rana nigromaculata*, inhabiting rice fields in Kyoto, Japan. Copeia 1999: 940–947.

HIRAI, T. AND M. MATSUI. 2000a. Myrmecophagy in a ranid frog *Rana rugosa*: Specialization or weak avoidance to ant eating? Zool. Sci. 17: 459–466.

HIRAI, T. AND M. MATSUI. 2000b. Feeding habits of the Japanese tree frog, *Hyla japonica*, in the reproductive season. Zool. Sci. 17: 977–982.

HIRAI, T. AND M. MATSUI. 2001a. Food habits of an endangered Japanese frog, *Rana porosa brevipoda*. Ecol. Res. 16: in press.

HIRAI, T. AND M. MATSUI. 2001b. Food partitioning between two syntopic ranid frogs, *Rana nigromaculata* and *R. rugosa*. Herpetol. J. 11(3): 109–115.

HIRAI, T. AND M. MATSUI. 2001c. Attempts to estimate the original size of partly digested prey recovered from stomachs of Japanese anurans. Herpetol. Rev. 32: 14–16.

HOUSTON, W. W. K. 1973. The food of the common frog, *Rana temporaria*, on high moorland in northern England. J. Zool., Lond. 171: 153–165.

INGER, R. F. AND B. GREENBERG. 1966. Ecological and competitive relations among three species of frogs (genus *Rana*). Ecology 47: 746–759.

JENSSEN, T. A. AND W. D. KLIMSTRA. 1966. Food habits of the green frog *Rana clamitans* in southern Illinois. Am. Midl. Nat. 76: 169–182.

KURAMOTO, M. 1967. Studies on *Rana limnocharis* Boie I. Population-hybrids and embryonic temperature tolerances. Bull. Fukuoka Univ. Educ. 3: 106–110.

LICHT, L. E. 1986. Food and feeding behavior of sympatric red-legged frogs, *Rana aurora*, and spotted frogs, *Rana pretiosa*, in southwestern British Columbia. Can. Field-Nat. 100: 22–31.

LIU, C. AND K. CHEN. 1933. Analysis of the stomach contents of two species of frogs (*Rana limnocharis* and *Rana nigromaculata*) in the vicinity of Kashing with special reference to insects. 1932 Year book, Bur. Ent. Hangchow: 183–191.

MCALPINE, D. F. AND T. G. DILWORTH. 1989. Microhabitat and prey size among three species of *Rana* (Anura: Ranidae) sympatric in eastern Canada. Can. J. Zool. 67: 2244–2252.

MAEDA, N. AND M. MATSUI. 1999. Frogs and Toads of Japan. Rev. Ed. Bun-ichi Sogo Shuppan, Tokyo. 221 p. (in Japanese with English abstract)

MILLER, J. D. 1978. Observations on the diets of *Rana pretiosa*, *Rana pipiens*, and *Bufo boreas* from Western Montana. Northwest Sci. 52: 243–249.

MOHANTY-HEJMADI, P. AND B. K. ACHARYA. 1982. Observation of food habits of six species of Indian frogs. J. Bombay. Nat. Hist. Soc. 79: 120–124.

OSAWA, S. 1998. On record of the introduced Indian rice frog (*Rana limnocharis*) in Kanai, Yokohama city. Bull. Kanagawa Pref. Nat. Conser. Center 15: 29–36. (in Japanese)

SHICHI, N., T. SERIZAWA, AND S. SERIZAWA. 1988. Growth and ovary development of *Rana limnocharis* in Kariya-Shi, Aichi Prefecture, central Japan. Jpn. J. Herpetol. 12: 95–101. (in Japanese with English abstract)

STEWART, M. M. AND P. SANDISON. 1972. Comparative food habits of sympatric mink Frogs, bullfrogs, and green frogs. J. Herpetol. 6: 241–244.

TOFT, C. A. 1985. Resource partitioning in amphibians and reptiles. Copeia 1985: 1–21.

TOMIDA, Y. 1976. Faunal survey of amphibians and reptiles of the southern hill area in Ueno city, Mie Prefecture, with some analytical notes on contents of their digestive organs. p. 143–181. In: A Report of Environmental Assesment and Influences by Urbanization in Soutern Part of Ueno City. Scientific reserch group of Mie Prefecture, Tsu. (in Japanese)

WERNER, E. E., G. A. WELLBORN, AND M. A. MCPEEK. 1995. Diet composition in postmetamorphic bull frogs and green frogs:

Implications for interspecific predation and competition. J. Herpetol. 29: 600-607.

WHITAKER, J. O. Jr., S. P. CROS, AND J. M. SKOVLIN. 1983. Food habits of the spotted frog *Rana pretiosa* from managed sites in Grant Country, Oregon, USA. Northwest. Sci. 57: 147–154.

Accepted: 9 November 2001