ACADEMIA JOURNAL OF BIOLOGY 2019, 41(3): 39–45
DOI: 10.15625/2615-0923/v41n3.13713

DIET OF *Odorrana chapaensis* (Bourret, 1937) FROM SON LA PROVINCE, VIETNAM

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Received 27 March 2019, accepted 28 May 2019

ABSTRACT

Since there is currently a lack of data on the natural history of *Odorrana chapaensis*, an amphibian species listed in the IUCN Red List (2019), we herein provided the feeding ecology of this amphibian species is virtually lacking. We herein provide data about the diet of *O. chapaensis* based on the results of our field work in Ngoc Chien Commune, Muong La District and Xim Vang Commune, Bac Yen District, Son La Province, Vietnam. We used the stomach-flushing method to obtain the stomach contents of 85 individuals at two survey sites. A total of 20 prey categories with 334 items, comprising 299 items of invertebrates and 35 unidentified items, were found in the stomachs of *O. chapaensis*. The dominant prey items of *O. chapaensis* were Araneae, Polydesmida, insect larvae, Blattodea, Coleoptera, Dermaptera, Lepidoptera, Hymenoptera, and Orthoptera. The importance index for these categories ranged from 3.5% to 32.5%. Coleoptera was the category with the highest frequency of prey items and its representatives were found in 45 stomaches.

Keywords: *Odorrana chapaensis*, food composition, prey items, stomach contents.

Citation: Pham Van Anh, Nguyen Quang Truong, Nguyen Van Hoang, 2019. Diet OF *Odorrana chapaensis* (Bourret, 1937) from Son La Province, Vietnam. *Academia Journal of Biology*, 41(3): 39–45. https://doi.org/10.15625/2615-0923/v41n3.13713.

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INTRODUCTION

Odorrana chapaensis was originally described from northern Vietnam by Bourret (1937). The species was listed in the IUCN Red List (2018) as Near Threatened (van Dijk et al., 2004). Recently, this species was recorded for the first time from Son La Province (Ngo et al., 2016). In Vietnam, this species is currently known from Lao Cai, Son La, and Ha Giang provinces (Nguyen et al., 2009, Ngo et al., 2016). Elsewhere, this species has been reported from China (Yunnan) (Nguyen et al., 2009). Information on the feeding ecology of *O. chapaensis* is virtually lacking. In this study, we investigated the dietary ecology of *O. chapaensis* from Son La Province.

MATERIALS AND METHODS

Field surveys were conducted in Son La Province from 27 to 30 April 2015, from 15 to 17 April 2016, from 27 to 30 April 2016, from 7 to 10 October 2016, and from 26 to 30 April 2017 by A. V. Pham, N. B. Song, H. V. Tu, T. V. Dau, H. A. Giang, and Q. T. Bui in Ngoc Chien Commune (21°21’46”N, 103°30’38”E, elevation: 1,780 m), Muong La District and Xim Vang Commune (21°36’378”N, 104°11’206”E, elevation: 1,730 m), Bac Yen District, Son La Province (fig. 1).

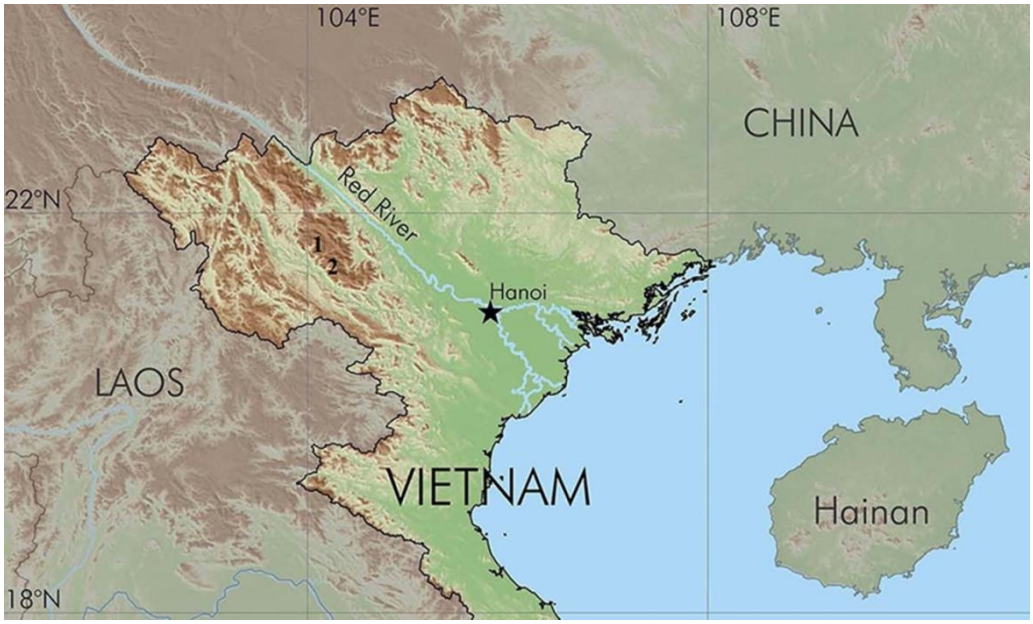

Figure 1. Map showing the survey sites in Son La Province: 1: Ngoc Chien; 2: Xim Vang

Specimens were collected by hand between 20:00 and 23:00. We measured snout-vent length (SVL) with a digital caliper to the nearest 0.1 mm. Based on the size of captured animals, we divided individuals into two age groups: adults with SVL > 50 mm and subadults with SVL ≤ 50 mm. We adopted stomach-flushing to obtain stomach contents without sacrificing frogs (Solé et al., 2005). Spatula, forceps, two syringes with thread (60 ml), and the infusion tube of soft material (silicon) were used to collect prey items in the stomach of frogs, with special attention to avoid perforations of the oesophagus and stomach of small individuals. In addition, some other tools (i.e., sieve, small airtight vials, and vessel) and 70% ethanol were used for filtration, storage, to contain waste water, and to preserve items. Each amphibian received stomach-flushing only once following the guidelines approved by the American Society of Ichthyologists and Herpetologists for Animal Care (Beaupre et al., 2004). The water for flushing was taken

from the streams where the frogs were captured and used after filtration. After flushing, frogs were monitored for the vital and body conditions and released within 30 min at the place of capture. Stomach samples were preserved in 70% ethanol for further analysis, and subsequently kept at the Department of Zoology, Faculty of Biology and Chemistry, Tay Bac University (TBU). For the taxonomic identification of frogs, we collected six voucher specimens for morphological analysis.

Prey items were identified under a microscope (Olympus SZ 700) based on identification keys (i.e., Csiro, 1991; Millar et al., 2000; Thai, 2003; Johnson &Triplehorn, 2005). The maximum length (L) and width (W) of each prey item were measured to the nearest 0.01 mm using either a caliper or a calibrated ocular micrometer fitted to a microscope. The volume (V) of prey items was calculated using the formula for a prolate spheroid (π = 3.14, Magnusson et al., 2003): $V = 4\pi/3 \times (L/2) \times (W/2)^2$ (mm^3). The index of relative importance (I) was used to determine the importance of each food category. This index provides a more informed estimation of food item consumption than any of the three components alone by using the following formula: IRI = (%F + %N + %V)/3 (Caldart et al., 2012), where F is the frequency of stomachs that contain a particular food item and N is the total number of the food items in relation to all food items. We used the reciprocal Simpson's heterogeneity index, 1/D, to calculate dietary heterogeneity: $D = \sum[ni(ni - 1)]/[N(N - 1)]$. Where ni is the number of food items in the *i*th taxon category and N is the total number of prey categories (Krebs, 1999).

To estimate prey evenness, we used Shannon's index of evenness. Evenness is calculated from the equation: J' = H'/Hmax = H'/ln S. The maximum diversity (Hmax) that could possibly occur is that which would be found in a situation in which all taxa had equal abundance (H' = Hmax = lnS), S is the total number of prey taxa, and H' is the Shannon - Weiner index of taxon diversity. The value of H' is calculated from the equation: $H' = -\sum(Pi \times \ln Pi)$, where the quantity Pi is the proportion of total food items belonging to the *i*th taxon for the total food items of the sample (Magurran, 2004; Muñoz-Pedreros & Merino, 2014).

Statistic analyses were performed with the SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) software and with the significance level set to $P < 0.05$ for all analyses. We used one-way analyses of variance (ANOVAs) to examine the number of prey items, frequency of occurrence, and prey volume collected among sex/age groups, localities, and months.

RESULTS AND DISCUSSION

A total of 85 stomachs of *O. chapaensis* from two sites in Son La Province were examined. Twelve of them (or 14.1%) were empty. The numbers of stomachs for sex and age groups were 69 adults (29 males, 40 females) and 16 sub-adults. For localities, 57 were collected in Ngoc Chien and 28 in Xim Vang. For the time of collection, 66 were in April and 19 in October (table 1).

A total of 334 prey items (299 items of invertebrates and 35 unidentified items) belonging to 20 categories was found in the stomachs of *O. chapaensis*. The numbers of prey items in males were 1–12 (average 6.08 ± 3.31 items) and 1–10 in females (average 6.08 ± 3.31 items) and 1–8 in sub-adults (3.6 ± 2.01 items). These values were significantly different (ANOVA; $F_{2, 72} = 4.81$; $P = 0.011$) between adults and subadults. The numbers of food items in the specimens from Ngoc Chien ranged from 1 to 12 (4.58 ± 3.23 items) and those from Xim Vang ranged 1–10 (4.56 ± 2.63 items).

The numbers from the two sites were not significantly different from each other ($F_{1, 72} = 0.0001$; $P = 0.98$). The numbers of food items collected in April (4.54 ± 3.07 items, ranging from 1 to 12) and in October (4.71 ± 2.97 items, ranging from 1 to 9) also were not significantly different from each other ($F_{1, 72} = 0.36$; $P = 0.85$).

Table 1. Dietary composition of *O. chapaensis* in Son La Province: F = frequency of occurrence, N = number of items, V = volume (mm^3), Ix = index of relative importance of each taxon (n = 73 stomach contents)

Prey category	Frequency (F)		Count (N)		Volume (V)		Ix
	F	%F	N	%N	V	%V	
Mollusca	3	1.59	3	0.90	298.03	0.48	0.99
Opiliones	3	1.59	3	0.90	72.68	0.12	0.87
Araneae	11	5.82	12	3.59	1,065.84	1.70	3.70
Polydesmida	11	5.82	17	5.09	495.52	0.79	3.90
Blattodea	9	4.76	9	2.69	10,340.80	16.50	7.99
Coleoptera	45	23.81	125	37.43	22,633.44	36.12	32.45
Dermaptera	9	4.76	11	3.29	1,632.64	2.61	3.55
Diptera	2	1.06	3	0.90	133.45	0.21	0.72
Ephemeroptera	1	0.53	2	0.60	68.06	0.11	0.41
Phasmatodea	1	0.53	1	0.30	778.46	1.24	0.69
Lepidoptera	16	8.47	34	10.18	6,298.41	10.05	9.57
Mantodea	1	0.53	1	0.30	2,009.60	3.21	1.35
Hymenoptera	17	8.99	24	7.19	1,291.77	2.06	6.08
Trichoptera	2	1.06	9	2.69	814.63	1.30	1.68
Hemiptera	4	2.12	5	1.50	353.14	0.56	1.39
Orthoptera	11	5.82	13	3.89	7,298.51	11.65	7.12
Plecoptera	1	0.53	1	0.30	70.65	0.11	0.31
Isoptera	3	1.59	3	0.90	69.36	0.11	0.87
Insecta larvae	18	9.52	23	6.89	3,839.48	6.13	7.51
Unidentified items	21	11.11	35	10.48	3,091.35	4.93	8.84
Total	189	100.00	334	100.00	62,655.82	100.00	100.00

The mean number of food items per individual was 4.58 ± 3.03 (ranging from 1 to 12; n = 73) and the mean volume per stomach was 858.3 ± 1098.54 mm^3 (ranging from 9.42 to 5091.51 mm^3). Mean food item length was 11.52 ± 7.75 mm (ranging from 1.0 to 60.0 mm, n = 334), mean food item width was 4.0 ± 2.34 mm (ranging from 0.2 to 14.0 mm, n = 334), and mean volume per food item was 187.59 ± 393.84 mm^3 (ranging from 0.13 to 3,590.07 mm^3).

We found 20 prey categories of invertebrates and only one category of unidentified items (table 1). The total dietary breadth of *O. chapaensis* from Northwest Vietnam was 0.18 (Simpson's index of diversity) and Shannon's evenness was 0.74. Average food volumes of adult male individuals (1414.92 ± 42.55 mm^3, ranging from 42.55 mm^3 to 5,091.51 mm^3), adult female individuals (840.65 ± 840.65 mm^3, ranging from 16.75 mm^3 to 3,215.56 mm^3) and subadults (87.77 ± 55.93 mm^3, ranging from 9.4242 mm^3 to 164.63 mm^3) were significantly different (ANOVA; $F_{2.72} = 6.36$; $P = 0.03$; fig. 2) from each other.

The average volume of prey items per stomach from Ngoc Chien (1057.15 ± 1244.42 mm^3, ranging from 9.42 mm^3 to 5,091.51 mm^3) and from Xim Vang (426.02 ± 463.8 mm^3, ranging from 23.55 mm^3 to

1,471.78 mm^3) were significantly different from each other (ANOVA: $F_{1.72} = 5.53$; $P < 0.02$).

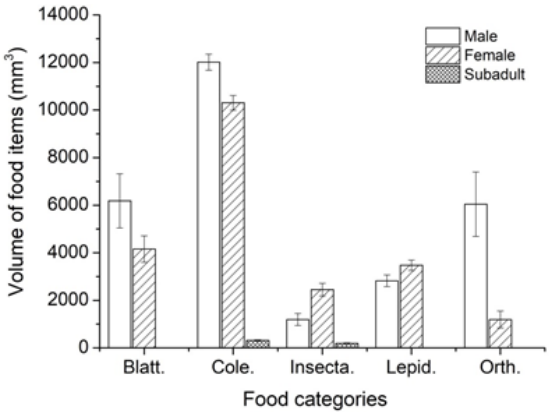

Figure 2. Volumes of major prey items (mean ± SD) of *O. chapaensis* from Son La Province: Cole = Coleoptera; Derma = Dermaptera; Insecta = Insect larvae; Ort = Orthoptera

The average volume of prey items per stomach in April (845.25 ± 1142.58 mm^3, ranging from 9.42 mm^3 to 1,142.58 mm^3) was not significantly different from the average prey volume per stomach in October (913.28 ± 924.79 mm^3, ranging from 42.55 mm^3 to 3,215.55 mm^3; $F_{1.72} = 0.043$; $P < 0.84$).

The population of *O. chapaensis* from the Ngoc Chien area consumed the highest number of food categories with 20 types, while the population from Xim Vang area only consumed 11 food types. However, the largest dietary breadth was found in Xim Vang (6.41), with an evenness of 0.84. In contrast, the narrowest dietary breadth was found in Ngoc Chien (4.75) with an evenness of 0.70 (table 2).

The number of food categories in April (18 types) was more diverse than that in October (15 types). The largest dietary breadth was found in October (5.66), with an evenness of 0.70, whereas the narrowest dietary breadth was found in April (4.99) with an evenness of 0.70 (table 2).

Table 2. Simpson's Index of Diversity and Shannon's Evenness among sex/age groups, sites, and month in the diet of *O. chapaensis* from Son La Province, Vietnam

Contents	Simpson's index 1/*D*	Shannon's evenness *E*
Adult males	5.29	0.77
Adult females	5.55	0.77
Subadults	6.56	0.87
Ngoc Chien	4.75	0.70
Xim Vang	6.41	0.84
April	4.99	0.70
October	5.66	0.70
Total	39.21	5.35

Adult males (17 prey categories) and females (16 prey categories) consumed more diverse prey than subadults (nine prey categories). However, the index of diversity of prey categories was higher in subadults (6.56 with an evenness index of 0.87) than in adult females (5.55 with an evenness index of 0.77) or adult males (5.29 with an evenness index of 0.77). Prey category richness from mid-curve points to late-curve points was higher for adult males and females than for subadults in both frequency (fig. 3a) and number of items (fig. 3b).

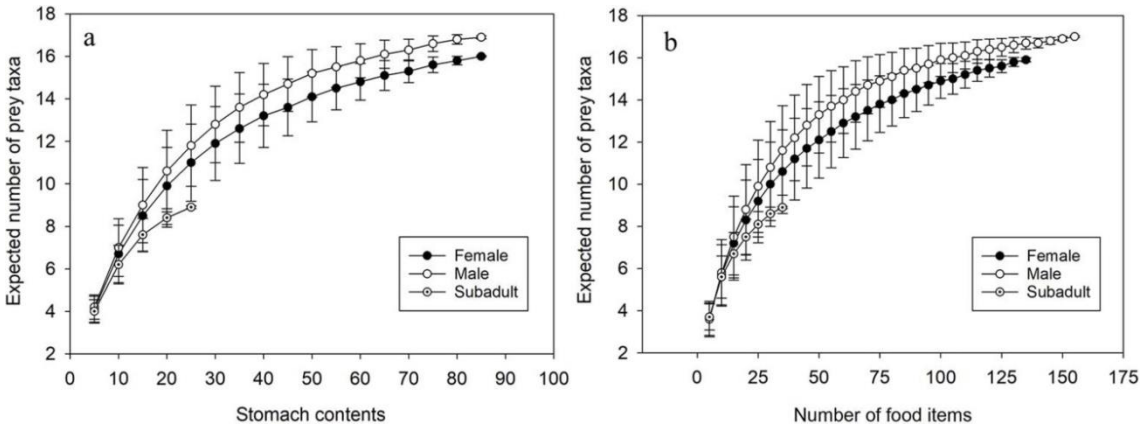

Figure 3. Expected prey-taxon accumulation curves from the data of (a) stomach contents and (b) counted food items consumed by *O. chapaensis* from Son La Province

CONCLUSION

A total of 20 prey categories with 334 items, comprising 299 items of invertebrates and 35 unidentified items, were found in the stomachs of *O. chapaensis*.

The dominant prey items of *O. chapaensis* were Araneae, Polydesmida, insect larvae, Blattodea, Coleoptera, Dermaptera, Lepidoptera, Hymenoptera, and Orthoptera, with importance indices ranging from 3.5% to 32.5%. The category of Coleoptera had the highest frequency of prey items and was found in 45 stomachs.

Ngo et al. (2014) studied the dietary composition of *Quasipaa verrucospinosa*, another anuran species from Central Vietnam. The prey categories of this species were more diverse than those of *O. chapaensis* (27 vs. 20 in the latter). Interestingly, prey of *Q. verrucospinosa* comprised mainly invertebrates, but also fishes, frogs and conspecific subadults (see Ngo et al. 2014), which were not found in *O. chapaensis* from Son La Province in this study. Even so, the major prey items of *Q. verrucospinosa* were relatively similar to those of *O. chapaensis*, consisting of Araneae, Coleoptera, Hymenoptera, and Decapoda (Ngo et al. 2014).

Pham & Nguyen (2018) recently reported the dietary composition of 23 prey categories of *Nanorana yunnanensis* from Son La Province. The major prey items of this frog species were Araneae, Coleoptera, Lepidoptera, Hemiptera, Orthoptera, and insect larvae. The food spectrum of *N. yunnanensis* slightly differed from that of O. *chapaensis* in having prey items of Diplura, Crustacea and Amphibia (see Pham & Nguyen, 2018).

Acknowledgements: We are grateful to the directorate of the Muong La Nature Reserve for the kind support for our field work. We thank Dau Van Trieu, Sung Ba Nenh and Bui The Quyen (Tay Bac University) for their assistance in the field. This research is supported by the Ministry of Education and Training (Grant No. B2019-TTB-562-13) to Pham Van Anh. Field equipment was donated by Idea Wild to Pham Van Anh.

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