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DIET OF THE INDOCHINESE WATER DRAGON *Physignathus cocincinus* CUVIER, 1829 (SQUAMATA: SAURIA: AGAMIDAE) FROM THUA THIEN HUE PROVINCE, VIETNAM

Nguyen Van Hoang,[1] Ngo Van Binh,[1]* Ngo Dac Chung,[1] and Nguyen Quang Truong[2]*

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The Indochinese Water Dragon, *Physignathus cocincinus* Cuvier, 1829, was listed in the Vietnam Red Data Book (2007) as Vulnerable. However, the knowledge about feeding ecology of this lizard species is still lacking. We herein provide data about the diet of *P. cocincinus* based on results of our field work in A Luoi District, Thua Thien Hue Province, Vietnam. We used the stomach-flushing method to obtain the stomach contents of 187 individuals at three survey sites. A total of 20 prey categories with 3066 items, comprising 3052 items of invertebrates and 14 items of plants, was found in the stomachs of *P. cocincinus*. The most common prey items of *P. cocincinus* were spiders, ants, insect larvae, termites, crickets, grasshoppers, and earthworms with the importance indices ranged from 5.36% to 35.57%. The total volume of food items consumed by adult and subadult individuals was greater than that consumed by juveniles ($P = 0.005$). The rarefaction curves for prey category richness were higher in subadults and in juveniles than that in adult individuals for both occurrence frequency and counted items.

Keywords: *Physignathus cocincinus*; food composition; prey items.

INTRODUCTION

The Indochinese Water Dragon, *Physignathus cocincinus* Cuvier, 1829, was originally described from southern Vietnam (Cuvier, 1829). This is a widely distributed species in tropical forests from southern China through Vietnam and Laos southwards to Thailand (Nguyen et al., 2009). This species was listed in the Vietnam Red Data Book (2007) as Vulnerable due to the population reduction and a decline in area of extent of occurrence and habitat quality (Dang et al., 2007). In Thua Thien Hue Province, *P. cocincinus* has been recorded in the evergreen forests of A Luoi, Huong Thuy, Huong Tra, Phu Loc, and Nam Dong districts (Nguyen et al., 2009). However, the population of *P. cocincinus* is under threatened because of habitat loss and overexploitation for food consumption and traditional medicine.

In Vietnam, several studies on biological and ecological characters of *P. cocincinus* have been undertaken, for instance, Ngo and Bui (2009) reported about ecological characters of the species in captivity; Ngo et al. (2016) provided some biological data of microhabitat use and foraging mode of *P. cocincinus* in Thua Thien Hue Province. Nguyen et al. (2017) analyzed day and night activities of *P. cocincinus* in Thua Thien Hue Province. Up to date, no data about the dietary composition of *P. cocincinus* in the natural habitat is available. Therefore, this study provides the first data about the diet of *P. cocincinus* from Thua Thien Hue Province.

MATERIAL AND METHODS

Field surveys were conducted in the dry season (from March to July) and the rainy season (from August to October) in 2015 at Thua Thien Hue Province. Stomach contents of *P. cocincinus* were collected in forest streams at three sites: Huong Nguyen forest (16°09′26″ N 107°26′58″ E; 175 m a.s.l.), Khe Dau forest (16°05′13″ N 107°28′66″ E; 694 m a.s.l.), and A Pat forest (16°04′59″ N 107°28′76″ E; 741 m a.s.l.) within

[1] University of Education, Hue University, 34 Le Loi Road, Hue, Vietnam.

[2] Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam; Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam.

* Corresponding authors: nvb6868@gmail.com, nqt2@yahoo.com

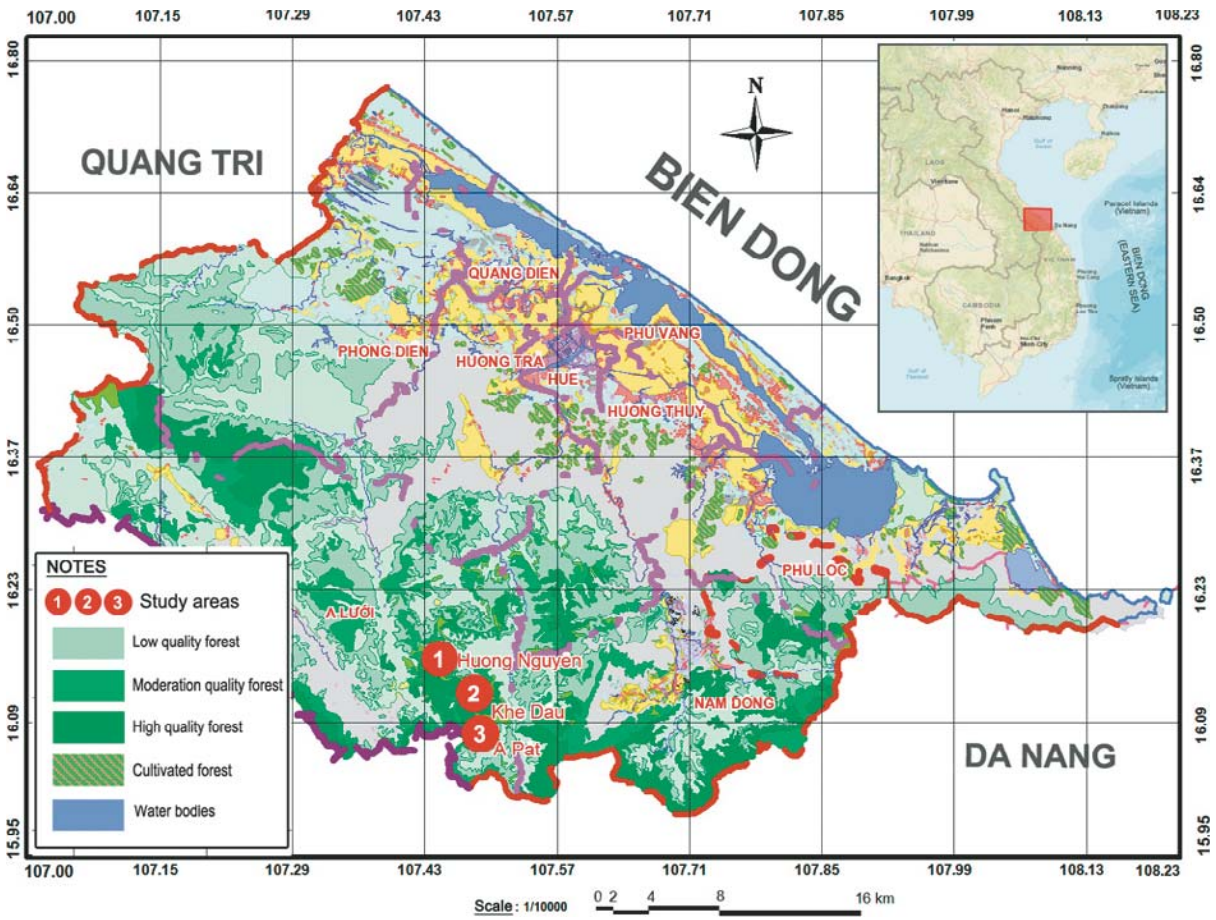

Fig. 1. Map showing the survey sites in Thua Thien Hue Province: 1, Huong Nguyen; 2, Khe Dau; 3, A Pat.

the Sao La Conservation Area, A Luoi District, Thua Thien Hue Province (Fig. 1).

The two-night surveys were conducted a long three streams in every month from March to October 2015 by a team of three people from 18:30 to 24:00. We visually searched for the Indochinese Water Dragons using spotlights, in water, on tree trunks and vegetation, and up to 15 m away from the stream over a transect length of 3 km. Specimens were collected by hand or noose. We measured snout-vent length (SVL), tail length (TaL), size of head with a measuring tape and body mass (BM) with an electronic balance. Based on the size and captured animals, we divided individuals into three age groups: adults with SVL > 130 mm, subadults with SVL from 100 – 130 mm, and juveniles with SVL < 100 mm.

For collecting stomach contents, we kept the animal individually in labeled bags for 30 min after capturing (Caldwell, 1996). We adopted the stomach-flushing method of Sole et al. (2005) to obtain stomach contents without sacrificing lizards. Soft catheter tubes of different sizes (inner diameter of 2, 3, or 5 mm) were used with appropriately sized syringes. Each lizard was stomach-flushed only once following the guidelines approved by the American Society of Ichthyologists and Herpetologists for animal care (Beaupre et al., 2004). After flushing, lizards were monitored for vigor and body conditions and released within 90 min at the place of capture. Seven specimens of *P. cocincinus* and stomach samples were preserved in 70% ethanol and deposited in the collection of the Faculty of Biology, University of Education, Hue University.

Prey items of each stomach were identified to the order level but some of them could be determined to the family level. For taxonomic identification of invertebrates, we followed Thai (2003) and Johnson and Triplehorn (2005). The length (head to thorax) and width (at widest centrally located section of body) of the body of each prey item were measured with a digital caliper to the nearest of 0.1 mm.

TABLE 1. The Dietary Composition of *P. cocincinus* (n = 187 stomachs) in Percentage Frequency of Occurrence (F), Number of Items (N) and Volume (V, mm^3), and the Index of Relative Importance (IRI) of Each Prey Category from Thua Thien Hue Province, Vietnam

Prey category	N	N%	F	F%	V	V%	IRI
Achatinidae	52	1.70	52	8.52	19,239.8	3.38	4.53
Araneae	42	1.37	41	6.72	45,521.4	8.00	5.36
Blattodea	14	0.46	14	2.30	13,166.9	2.31	1.69
Coleoptera	26	0.85	26	4.26	16,118.9	2.83	2.65
Decapoda	1	0.03	1	0.16	9382.2	1.65	0.61
Dermaptera	8	0.26	8	1.31	3534.6	0.62	0.73
Diptera	2	0.07	2	0.33	535.2	0.09	0.16
Formicidae	562	18.33	99	16.23	42,324.8	7.44	14.00
Hemiptera	5	0.16	5	0.82	3046.5	0.54	0.51
Hymenoptera(others)	25	0.82	25	4.10	14,742.3	2.59	2.50
Insect larvae	77	2.51	77	12.62	27,589.6	4.85	6.66
Isoptera	2069	67.48	102	16.72	128,067.5	22.50	35.57
Julidae	24	0.78	10	1.64	22,531.4	3.96	2.13
Lepidoptera	33	1.08	30	4.92	12,328.7	2.17	2.72
Lumbriculida	19	0.62	19	3.11	86,184.9	15.14	6.29
Neuroptera	12	0.39	12	1.97	6955.2	1.22	1.19
Orthoptera	53	1.73	51	8.36	101,534.3	17.84	9.31
Phasmatodea	1	0.03	1	0.16	246.2	0.04	0.08
Plants	14	0.46	8	1.31	2969.8	0.52	0.76
Unidentified	27	0.88	27	4.43	13,177.7	2.32	2.54
Total	3066	100	610	100	569,197.9	100	100

Digested items that could not be identified were considered as unidentified prey. Volume of each prey item (V) was estimated by the formula for a prolate spheroid (see Biavati et al., 2004; Ngo et al., 2014):

$$V = \frac{4}{3}\pi \frac{\text{length}}{2}\left(\frac{\text{width}}{2}\right)^2.$$

We also calculated the percentage of frequency of occurrence (F) (or percentage of stomachs containing each prey category) and the numeric percentages (N) of each prey item in relation to all the prey items. We used the Index of Relative Importance (IRI) to determine the importance of each prey category in the diet of *P. cocincinus* (Biavati et al., 2004; Caldart et al., 2012):

$$\text{IRI} = \frac{F\% + N\% + V\%}{3},$$

where IRI is relative importance index for each prey category, F% is occurrence percentage, N% is numeric percentage, and V% is volumetric percentage.

We use the reciprocal Simpson's heterogeneity index, $1/D$, to calculate dietary heterogeneity, where

$$D = \frac{1}{N(N-1)}\sum n_i(n_i - 1),$$

n_i is the number of prey items in the ith prey category and N is the total number of prey categories (Krebs, 1999).

To estimate prey evenness, we used Shannon's evenness. Evenness is calculated from the equation $J' = \frac{H'}{H_{\max}} = \frac{H'}{\ln S}$, where the maximum diversity ($H_{\max}$) that could possibly occur where all taxa have equal abundance ($H' = H_{\max} = \ln S$), S is the total number of prey taxa and H' is the Shannon – Weiner index of taxon diversity. Here, the value of H' is calculated from the equation

$$H' = -\sum (p_i \ln p_i),$$

where the quantity p_i is the proportion of total food items belonging to the ith taxon for the total food items of the sample (Magurran, 2004; Muñoz-Pedreros and Merino, 2014).

The rarefaction method was used to estimate the diversity of diet among adults, subadults, and juveniles. We standardized all diet samples from the rarefaction method using the modified algorithm by Hurtlbert (1971) and Simberloff (1972) as follows:

$$E(S_n) = \sum_{i=1}^{S}\left(1 - \frac{K_n^{N-N_i}}{K_n^N}\right),$$

where $E(S_n)$ is the expected number of prey taxa in a random sample of n prey items, S is the total number of prey taxa in the entire collection from stomach contents, N_i is the number of prey items in the ith taxon, N is the total number

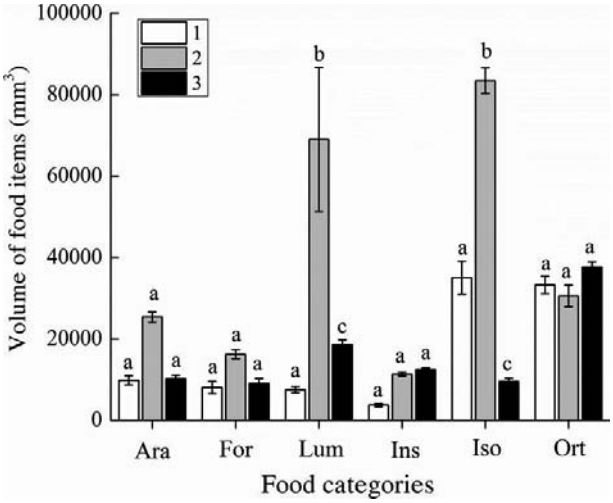

Fig. 2. Volumes of major prey items (mean ± SD) of *P. cocincinus* (1, adults; 2, subadults; 3, juveniles) from Thua Thien Hue Province: Are, Araneae; For, Formicidae; Lum, Lumbriculida; Ins, Insect larvae; Iso, Isoptera; Ort, Orthoptera. Different letters above the three age groups for the same food item indicated a significant difference ($P < 0.05$) in volumes of prey.

of prey items in the entire collection ($\sum N_i$), n is the value of sample size (number of prey items) chosen for standardization ($n \leq N$) and K_n^N is the number of combinations of n prey items that can be chosen from a set of N prey items (Krebs, 1999).

Statistic analyses were performed with the SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA) for Windows 10 and set the significance level to $P \leq 0.05$ 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 age groups, localities, and seasons. Chi-squared tests were used to examine the number of specimens (stomach contents) collected among age groups, localities, and seasons.

TABLE 2. Simpson's Index of Diversity and Shannon's Evenness Among Age Groups, Sites, and Seasons in the Diet of *P. cocincinus* from Thua Thien Hue Province, Vietnam

Contents	Simpson's index $1/D$	Shannon's evenness E
Adults	2.39	0.23
Subadults	1.57	0.12
Juveniles	2.99	0.22
Huong Nguyen	2.08	0.10
Khe Dau	1.44	0.12
A Pat	3.11	0.26
Dry season	2.50	0.18
Rainy season	1.59	0.14
Total	2.04	0.15

RESULTS AND DISCUSSION

A total of 211 stomachs of *P. cocincinus* from three sites in Thua Thien Hue Province was examined for the contents. Twenty-four of them (or 11.5%) were empty. The numbers of collected stomachs for age groups (43 adults, 70 subadults, 98 juveniles; $\chi^2 = 11.24$; $df = 14$; $P = 0.667$) and localities (122 in Huong Nguyen, 35 in Khe Dau, 54 in A Pat; $\chi^2 = 15.65$; $df = 14$; $P = 0.335$) were not significantly different. However, those between seasons (120 in the dry season and 91 in the rainy season) were significantly different ($\chi^2 = 37.17$; $df = 4$; $P < < 0.0001$).

A total of 3066 prey items (3052 items of invertebrates and 14 items of plants) belonging to 20 categories was found in the stomachs of *P. cocincinus* (Table 1). The numbers of food items in adults (average 11.7 ± 2.5 items, ranging from 1 to 93), in subadults (25.2 ± 5.4 items, ranging from 1 to 238) and in juveniles (11.1 ± 2.6 items, ranging from 1 to 166) were significantly different (ANOVA; $F_{2,186} = 4.155$; $P < 0.01$). The mean numbers of food items from Huong Nguyen (16.6 ± 2.9 items), from Khe Dau (24.9 ± 9.7 items), and from A Pat (10.2 ± ± 2.1 items) were not significantly different ($F_{2,186} = = 1.952$; P? 0.1). The numbers of food items in the dry season (14.4 ± 1.8 items, ranging from 1 to 93) and in the rainy season (18.8 ± 4.7 items, ranging from 1 to 238) were also not significantly different ($F_{1,186} = 3.577$; $P = = 0.06$).

The mean number of food items per individual was 16.4 ± 2.4 (ranging from 1 to 238; $n = 187$) and the mean volume per stomach was 3043.8 ± 326.2 mm^3 (ranging from19.1 to 34, 964.6 mm^3). Mean food item length was 10.9 ± 8.4 mm (ranging from 3.3 to 165.5 mm; $n = = 3066$), mean food item width was 3.1 ± 2.4 mm (ranging from 0.6 to 31.4 mm; $n = 3066$), and mean volume per food item was 185.7 ± 18.5 mm^3 (ranging from 0.7 to 33, 184.1 mm^3). We found 19 prey categories of animals and one category of plants (Table 1), and the total dietary breadth of *P. cocincinus* from central Vietnam was 2.04 (Simpson's index of diversity); Shannon's evenness was 0.15. Average food volumes of each adult individual (3419.7 ± 588.7 mm^3), subadult individual (4179.9 ± ± 757.7 mm^3) and juvenile (1814.6 ± 210.1 mm^3) were significantly different (ANOVA; $F_{2,186} = 5.496$; $P = = 0.005$; Fig. 2).

The average volumes of prey per stomach from Huong Nguyen (3131.3 ± 497.6 mm^3), from Khe Dau (3163.1 ± 531.5 mm^3), and from A Pat (2695.3 ± ± 482.4 mm^3) were not significant difference (ANOVA: $F_{2,186} = 0.173$; $P > 0.8$). Average volumes of each stom-

ach between dry and rainy seasons also were not significantly different (dry season: 2486.1 ± 323.8 mm^3; rainy season: 3742.8 ± 623.7 mm^3; $F_{1,186} = 0.861$; $P > 0.3$). The largest dietary breadth was found in A Pat (3.11) with an evenness of 0.26; whereas the narrowest dietary breadth was found in Khe Dau (1.44) with an evenness of 0.12 (Table 2).

Although the index of diversity of prey categories was higher in juveniles (2.99 with an evenness index of 0.22) than in adults (2.39 with an evenness index of 0.23) and in subadults (1.57 with an evenness index of 0.12), prey category richness at mid-curve points to late-curve points was higher for subadults and juveniles than for adults in both frequency (Fig. 3A) and number of items (Fig. 3B). Using the rarefaction curves for prey taxon richness allowed a comparison between the expected number of prey taxa in a fixed number of prey categories by moving vertically in Fig. 3 at any fixed number of prey items. The comparison can be taken at the point at which the abundance level of the larger sex matches the level in the smaller one.

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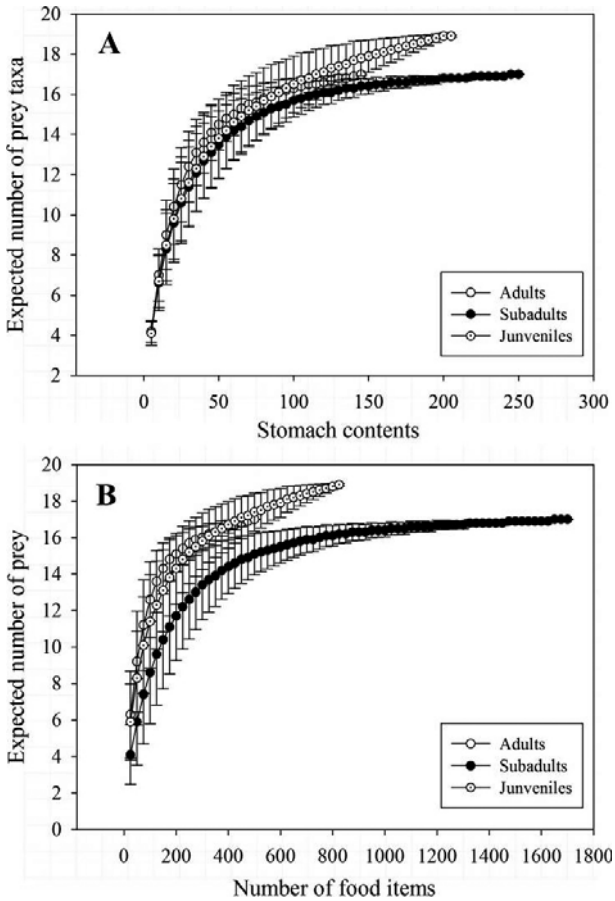

Fig. 3. Expected prey-taxon accumulation curves from the data of (A) stomach contents and (B) counted food items consumed by *P. cocincinus* from Thua Thien Hue Province. Circles represent the expected mean values, and the graphs showed the 95% confidence intervals for three age groups.

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