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Diet of the Bannan Caecilian *Ichthyophis bannanicus* (Amphibia: Gymnophiona: Ichthyophiidae) in the Mekong Delta, Vietnam

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ABSTRACT.—*Ichthyophis bannanicus* is a terrestrial caecilian, and little is known about many aspects of the ecology of this species. We investigated the correlation between diet and body size, spatiotemporal variation in dietary composition, diversity index of prey, and size dimorphism among populations for this species. Specimens ($N = 135$) were collected from May 2010 to April 2011 in the Mekong Delta, Vietnam. We found 178 prey items representing 11 unique families and discovered that the diet of *I. bannanicus* is composed mainly of small prey; the most important were Achatinidae, Lumbricidae, and Formicidae, with a combined importance index of 77%. Diet composition, prey size, and total prey volume in *I. bannanicus* changed between dry and rainy seasons and among regions ($P < 0.001$). Mean prey volume increased with body size and showed positive correlations ($P < 0.001$) between total length and mouth width and prey size consumed. The total dietary breadth of *I. bannanicus* was 4.52, and all six populations of the Bannan Caecilian were found to consume snails, earthworms, ants, and termites. Results indicated that precipitation, but not temperature, was associated positively with prey volume. All collected *I. bannanicus* were adult females; we did not find male individuals of this species in the study area.

Amphibian species are declining around the world (Kiesecker et al., 2001; Green, 2003; Storfer, 2003; Stuart et al., 2004; Becker et al., 2007), and a loss of diversity in amphibian species is expected to affect both aquatic and terrestrial food webs of tropical ecosystems (Houlahan et al., 2000; Kupfer et al., 2005b; Wells, 2007). The Mekong Delta is a region of high biodiversity in Indochina (Meynell, 2003; Dubeau, 2004) and is considered to be one of the biodiversity hotspots in the world (Myers et al., 2000). This region is affected strongly by habitat fragmentation or loss and climate change. In addition, droughts and floods are threats to amphibians in the Mekong Delta, Vietnam (Le et al., 2007; Hoang, 2012; Wu et al., 2013). Therefore, the resident Bannan Caecilian (*Ichthyophis bannanicus*) is listed in the Vietnamese Red List as a vulnerable species (VU) and should be protected. However, information on the feeding ecology of *I. bannanicus* is lacking. Thus, our study of diet, including an assessment of diversity in diet, is a valuable contribution to our understanding of this caecilian.

Currently, numerous amphibian species exist in Vietnam (approximately 200 species; BVN, pers. comm., unpubl. data) compared to the Indochina region. However, only one genus of caecilian has been reported from Indochina, and *I. bannanicus* is the only caecilian species present in Vietnam (Nguyen et al., 2009). Previous studies indicate that *I. bannanicus* is distributed in certain regions at different elevations and in various temperature and humidity regimes (Nguyen et al., 2005, 2009). The recent study on the molecular phylogeny of genus *Ichthyophis* in which specimens were collected from Southeast Asia found two distinct lineages in Indochina (Gower et al., 2002; Nishikawa et al., 2012). According to Nishikawa et al. (2012), one branch from northern Vietnam and Laos, northeastern Thailand, and southern China was identified as the Bannan Caecilian (*I. bannanicus*); the other is a new species, *Ichthyophis nguyenorum*, found in Kon Tum Plateau, central Vietnam.

According to Nguyen et al. (2005) Bannan Caecilians grow to 325–430 mm total length. Several previous investigations indicate that *I. bannanicus* is distributed commonly in riparian forests at elevations between 500 and 1,500 m a.s.l., at depths of 20–30 cm, and under leaf litter but mainly in humid soil near temporary ponds and close to rivers (Nguyen, 2005; Hoang, 2012). Some incomplete studies related to ecology, food habits, and microhabitat use of ichthyophiid caecilians with specimens collected from the Mekong valley, northeastern Thailand (Kupfer et al., 2005a,b) have been published, representing important progress toward the understanding of these amphibian species. Previous studies also indicate that most adult caecilians are terrestrial and well adapted to subterraneous life (Kamei et al., 2012; Hoang, 2012). However, little information is available on most aspects of their ecology (Kupfer et al., 2005b); thus, the dietary study of *I. bannanicus*, with their secretive lifestyle, is necessary to gain insight into their ecology and population status and to inform management and conservation plans.

In our study, we investigated the dietary composition of *I. bannanicus* living in the tropical region. We examined the spatiotemporal variation (among sites and seasons) in the feeding biology of *I. bannanicus* in different habitats of the fragmented secondary forest and compared it to the lowland in the Mekong Delta, Vietnam. We tested the hypothesis that differences in sampled sites result in different degrees of food use in this species. We predicted that the dietary composition of *I. bannanicus* is similar among seasons and that it consists mainly of three prey groups (megascolecid earthworms, ants, and termites) similar to what was reported for the diet of the terrestrial *Ichthyophis kohtaoensis* from the Isan Region of northeastern Thailand (Kupfer et al., 2005b). Also, we compared the diet of adult females to examine season-dependent dietary variation and test the notion that the size of prey consumed and the dietary breadth of *I. bannanicus* are correlated positively with their size-related morphometric measurements.

MATERIALS AND METHODS

Study Sites.—This study was conducted at Dong Thap (10°08′07″–10°58′21″N, 105°11′34″–105°56′35″E) and An Giang (10°10′55″–10°57′39″N, 104°46′40″–105°34′25″E), Provinces in the Mekong Delta, Vietnam (Fig. 1). Tropical climate and seasonal monsoons characterize this study area, with an annual average

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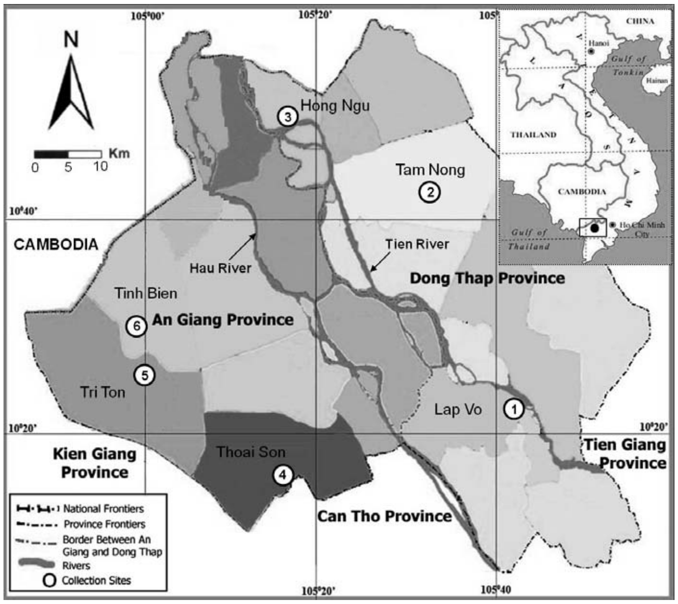

FIG. 1. Map of Dong Thap and An Giang Provinces showing the geographic location of the six localities where specimens (white circles) of *Ichthyophis bannanicus* were obtained: (1) Lap Vo, (2) Tam Nong, and (3) Hong Ngu Districts in Dong Thap Province; (4) Thoai Son, (5) Tri Ton, and (6) Tinh Bien Districts in An Giang Province in the Mekong Delta, Vietnam.

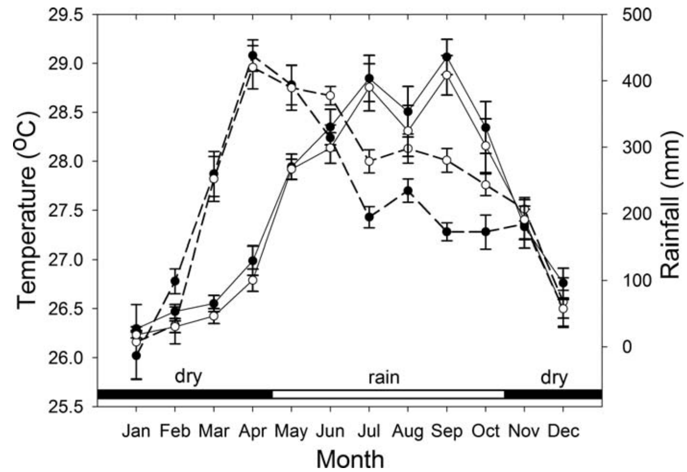

FIG. 2. Monthly mean precipitation (solid lines, in millimeters) and temperature (broken lines, in °C) in An Giang (filled circles) and Dong Thap (open circles) Provinces in the Mekong Delta, Vietnam, over the last 10 years. Data are presented as the mean ± SE, referred from weather stations in An Giang and Dong Thap Provinces (unpubl. data).

temperature of 27.62 ± 0.047°C (ranging from 26.098 ± 0.22°C in January to 29.02 ± 0.13°C in April) and an annual average rainfall of 2,528.44 ± 56.07 mm. A relatively dryer period with low rainfall extends from January to April and November to December each year, with monthly rainfall ranging from 22.5–185.6 mm (82.87 ± 24.29 mm). This is considered the dry season, whereas most rain is restricted to the wet season between May and October (approximately 6 months), with monthly rainfall ranging from 268.6–422.1 mm (342.91 ± 23.25 mm) (Fig. 2). The study area is dominated by secondary forests that are fragmented at elevations of 50–716 m a.s.l. Additionally, there are high mountains such as Cam Mountain, referred to as "house ridge" of the southwest in An Giang Province (Le, 2007). In contrast, the localities sampled in Dong Thap Province are mainly the plain regions, at elevations between 3 and 15 m a.s.l. (Weather stations in An Giang and Dong Thap Provinces, unpubl. data).

Sample Collection.—All specimens of *I. bannanicus* were collected at the following localities in Dong Thap (sites 1, 2, and 3) and An Giang (sites 4, 5, and 6) Provinces, the Mekong Delta, Vietnam: (1) Lap Vo (10°22′36″N, 105°42′48″E, at 7 m a.s.l., $N = 22$); (2) Tam Nong (10°42′41″N, 105°32′04″E, at 4 m a.s.l., $N = 26$); (3) Hong Ngu (10°49′21″N, 105°17′10″E, at 7 m a.s.l., $N = 21$); (4) Thoai Son (10°15′55″N, 105°16′35″E, at 53 m a.s.l., $N = 20$); (5) Tri Ton (10°26′43″N, 105°00′03″E, at 75 m a.s.l., $N = 20$); and (6) Tinh Bien (10°29′58″N, 104°59′26″E, at 476 m a.s.l., $N = 26$). Of these, Lap Vo, Tam Nong, and Hong Ngu Districts belong to the plain regions. The dominant vegetation in these regions is typical for the Mekong Delta and varies in form, ranging from dense grassland, usually with a sparse covering of shrubs and small trees, to ricefields, cornfields, sugar-cane fields, and marshes. The area is transversed by the of Tien and Hau River systems (Fig. 1). Thoai Son, Tri Ton, and Tinh Bien Districts are tropical mountain areas belonging to the Vietnamese southwest with fragmented secondary forests, which consist of a mosaic of riparian forest, mesophytic forest, eucalyptus culture, and open areas (tracks and glades).

Twice per month from May 2010 to April 2011 (total of 12 months), we collected 69 caecilian specimens from the Dong Thap (DT) and 66 specimens from the An Giang (AG) Provinces. All caecilians were adult females of *I. bannanicus* (sex of specimens was determined by examination of gonads and the presence of yolked follicles or oviductal eggs). We have not found male individuals of this species in the study area thus far. Specimens were collected in approximately equal proportions from the different locations and seasons (72 from the rainy season and 63 from the dry season). We conducted nocturnal surveys at each site (especially after rains) from 1800–2300 h daily. At these sites, we collected samples along lines, approximately 1.5–2.0 km in length. In the rainy season of 2010 (early May to late October), we sampled *I. bannanicus* mainly after rains, and we found most of adult females on the surface of the ground, whereas in the dry season (from November 2010 to late April 2011), we found *I. bannanicus* underground or under leaf litter. Animals were discovered by digging to a maximal depth of 45–55 cm with hoes. Collecting was conducted throughout the riparian forest, forestry plantations, and fields in humid soil near rivers or temporary ponds. We visually searched or dug for *I. bannanicus*, and the specimens were collected by hand and placed into individually labeled bags. The following variables were recorded for each collected individual: site, date, time, elevation, temperature, relative humidity, type of vegetation, and substrate type (leaf litter, moss, forest floor, and leaves). Monthly precipitation data were obtained from weather stations in DT and AG. For each sample site where caecilians were found, coordinates were taken with a GPS (datum WGS 84) to determine the distance among sites and the distribution of *I. bannanicus* in the Mekong Delta.

Individuals were measured with a measuring tape (to the nearest 0.1 mm) for total length (TL), whereas mouth width (MW) was measured with digital calipers to the nearest 0.01 mm, and body mass (BM) was determined with an electronic balance to the nearest 0.01 g. In the laboratory, all caecilians were euthanized humanely with a solution of MS-222 (Tricaine methanesulfonate) the same day that they were collected. Then stomach contents were separated and fixed in 10% formalin within two days (to preserve the stomach contents, fixation was usually carried out within 2 h after collecting). All stomach contents were preserved in 95% ethanol to interrupt the decomposition processes until they could be processed for later laboratory analyses. Some specimens and stomach contents were transferred to 70% ethanol and deposited in the herpetological collection of the Faculty of Biology, Dong Thap University, Vietnam.

Stomach Content Analysis.—We sorted and identified prey items in each stomach content sample to the lowest possible taxonomic level (mostly order, and family when possible) under a dissecting microscope (MSL4000 Standard Stereo Microscope, magnification of 10×). We consulted and followed keys and descriptions in Thai (2001) and Johnson and Triplehorn (2005). We measured the length (head to thorax) and width (at widest centrally located section) of the body of each prey item, or made a best estimation for incomplete items, with a digital caliper to the nearest of 0.01 mm. Materials, such as sand, stones, and vegetative parts were considered to be ingested accidentally and were excluded from the analysis.

All unidentified materials (e.g., digested items that could not be identified) in the diet of caecilians such as unidentified insects were considered in the analyses. We calculated the percent volume of each prey item and unidentified material and estimated the volume (V) by the formula for a prolate spheroid (Caldwell and Vitt, 1999; Biavati et al., 2004; Valderrama-Vernaza et al., 2009; Ngo et al., 2013):

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

We adopted the reciprocal Simpson's index, $B = 1/(\Sigma p_i^2)$, to calculate the dietary breadth of caecilians in the Mekong Delta, where i is the prey category and p is the proportion of prey in category i, and n is the total number of prey categories (Krebs, 1999; Magurran, 2004). Also, we calculated the percent frequency of occurrence (F), which is the 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 relative importance index (RI) to determine the overall importance of each prey category in the diet of caecilians. This quotient (RI) provides a more informed estimation of prey consumption than do any of the three components alone by using the following formula (Biavati et al., 2004; Leavitt and Fitzgerald, 2009):

$$RI = \frac{\%F + \%N + \%V}{3}$$

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

Data Analysis.—Unless otherwise noted, all data are presented as the mean ± standard error (SE), and proportional data were arcsine-transformed to meet the normality requirement (Rosner, 2010; Zar, 2010). We performed statistical analyses using SPSS 14.0 software (SPSS Inc., Chicago) and STATISTICA 10.0 (StatSoft Inc., Tulsa, OK) for Windows 7 and set the significance level to $P < 0.05$. Chi-squared tests were used to examine the number of specimens (stomach contents) collected among localities and seasons. We tested correlations between body size (TL, BM, and MW) and prey size (length, width, and volume) to see whether prey size depended on caecilian size. The possible effects of climatic factors on feeding ecology and prey volume were tested with multiple linear regressions between the monthly scores of rainfall (mm) and temperature (°C) and prey volume. Multiple regressions between prey size (length, width, and volume) and body size (TL, BM, and MW) were also used to examine significant effects of relationships among localities and seasons.

Heterogeneity measures of dietary ecology are those that combine the richness and evenness components of diversity for the community. To estimate dietary diversity of caecilians among localities and seasons, we used the Simpson index as follows: $D = \sum([n_i(n_i - 1)]/[N(N - 1)])$, where n_i is the number of prey items in the i^{th} prey category; and N is the total number of prey categories. As D increases, diversity of diet decreases; therefore, Simpson's index is used as $1/D$ in this study. Also, we used the converted Simpson index to estimate the heterogeneity (SH) of diet among localities and seasons. In there, the 95% confidence intervals (CI) of the former index were assessed by the equation such as $\Sigma\Phi_i/n \pm t_{0.05(n-1)}SE_\Phi$, by the jackknife method, where $\Phi_i = nSt - [(n - 1)St_{-i}]$, $SE_\Phi = \sqrt{\{\Sigma[\Phi_i - (\Sigma\Phi_i/n)]^2/n(n - 1)\}}$, St is the sample statistic of SH, St_{-i} is the sample statistic missing out sample i, and n is the number of samples (Magurran, 2004).

Dietary data of each month were grouped into two-month intervals that correspond to the two rainfall periods that occur during the year (Fig. 2). One-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) as a post-hoc comparison was used to test for TL, BM, and MW among localities or prey size among seasons and sites. An ANOVA also was employed to examine the total volume of prey categories, prey length, and prey width between seasons and sites. Statistical differences in nutritional activities among seasons and sites were assessed using analysis of covariance (two-factor ANCOVA) on the total volume of prey categories with total length (TL) and mouth width (MW) as a covariate (Rosner, 2010; Zar, 2010). Multivariate analysis of variance (two-factor MANOVA) with Wilks' λ values was used to examine the effects of factors such as seasons and sites on the variance in the relative proportions of each dominant prey category.

Results

We collected 135 female *I. bannanicus* from 6 localities in Dong Thap (Lap Vo, Tam Nong, and Hong Ngu Districts) and An Giang (Thoai Son, Tri Ton, and Tinh Bien Districts) Provinces. The number of females was not different among localities ($\chi^2 = 1.421$, df = 5, $P > 0.922$) or between seasons ($\chi^2 = 2.098$, df = 5, $P > 0.835$). On average, adult females ($N = 69$) in Dong Thap Province (Fig. 3) were larger in TL, BM, and MW than were adult females ($N = 66$) in An Giang Province (Fig. 4): TL, 29.49 ± 0.38 cm (range 24.5–35.1 cm) in Dong Thap Province and 25.36 ± 0.38 cm (range 20.7–33.1 cm) in An Giang Province (ANOVA, $F_{1,133} = 59.07$, $P < 0.001$); BM, 36.95 ± 1.14 g (range 24.07–52.71 g) in Dong Thap Province and BM, 27.24 ± 0.82 g (range 18.91–46.93 g) in An Giang Province (ANOVA, $F_{1,133} = 46.87$, $P < 0.001$); and MW, 8.88 ± 0.18 mm (range 6–12 mm) in Dong Thap Province; MW, 7.26 ± 0.13 mm (range 6–10 mm) in An Giang Province (ANOVA, $F_{1,133} = 52.59$, $P < 0.001$).

Less than 18% (24) of the 135 *I. bannanicus* stomachs were empty. One hundred and seventy-eight prey items were identified (105 from Dong Thap Province and 73 from An Giang Province); 74 prey items in the dry season and 104 prey items in the rainy season. Prey items represented 11 unique categories, mainly invertebrates but also some vertebrates (e.g., small frogs, Table 1). The mean number of prey items per individual was 1.32 ± 0.08 (range 0–4). Mean prey length was

FIG. 3. Adult female of *Ichthyophis bannanicus* Yang, 1985, in life, from the plain region of Lap Vo District in Dong Thap Province, the Mekong Delta, Vietnam (10°22′36″N, 105°42′48″E, at 7 m a.s.l. elevation; total length of 30.1 cm; body mass of 18.2 g). Photo by Nghiep T. Hoang.

FIG. 4. Adult female of *Ichthyophis bannanicus* Yang, 1985, in life, from a hilly region in Tinh Bien District, An Giang Province, the Mekong Delta, Vietnam (10°29′58″N, 104°59′26″E, at 476 m a.s.l. elevation; total length of 24.8 cm; body mass of 12.3 g). Photo by Binh V. Ngo.

10.77 ± 0.48 mm (range 4–36), mean prey width was 4.41 ± 0.11 mm (range 2–7), and average prey volume was 119.91 ± 8.31 mm^3 (range 8.37–678.2).

Eleven prey categories were represented, excluding unidentified materials, plants and stones (Table 2). The total dietary breadth of adult females from the Mekong Delta was 4.52. The broadest diets were from Hong Ngu District, Dong Thap Province (4.48), and Tinh Bien District, An Giang Province (5.96), whereas the narrowest diets were found at Lap Vo District (3.41) and Thoai Son District (3.79). The two populations with intermediate dietary breadths were at Tam Nong (4.01) and Tri Ton (4.27) Districts. The two populations of the Bannan Caecilian *I. bannanicus* at Hong Ngu and Tinh Bien Districts consumed 9 prey categories, whereas the two populations at Thoai Son and Lap Vo Districts consumed only of 4 and 5 prey categories, respectively. All six populations of *I. bannanicus* from the Mekong Delta consumed snails, earthworms, ants, and termites, with a total dietary breadth of approximately 89% (Table 2).

We estimated an overall SH index of 4.429 (SE = 0.654; CI: 3.776–5.084) for the Mekong Delta. Between the two localities, both SH and SE values were higher at An Giang Province (SH = 4.778 (SE = 2.426) than at Dong Thap Province (SH = 4.187 (SE = 0.836). As a result, the CI value varied more in An Giang Province (CI, 2.351–7.204) than in Dong Thap Province (CI, 3.351–5.023). However, the SH values between Dong Thap and An Giang Provinces were similar (difference of 0.591). The overall SH = 4.675 (SE = 1.916; CI: 2.759–6.591) for both dry and rainy seasons. When Simpson's index was applied for the diet of populations of adult females between seasons, the SH values were considerably higher in the rainy season (SH = 5.817; CI, 5.002–6.632) than in the dry season (SH = 4.126; CI, 2.111–6.141), whereas the SE values were dramatically higher in the dry season (SE = 2.015) than in the rainy season (SE = 0.815).

The dominant prey of *I. bannanicus* were snails, earthworms, and ants and accounted for 79.04% of frequency of occurrence, 79.21% of prey items, and 72.94% of the total volume and an overall importance index of 77.07% (Table 1; Fig. 5). The number of Achatinidae, Formicidae, and Lumbricidae were the most important prey. Araneae, Coleoptera, Diplopoda, and insect larvae were less important prey items, making up 4.49% of the total number of prey items and having a relative importance index of 5.01%. Remaining prey items such as Isoptera, Orthoptera, Ranidae, and Scolopendromorpha had an intermediate importance of 13.02%. In addition, 11 unidentified items were found in the *I. bannanicus* stomachs with a relative importance index of 4.9% (Table 1). Several root and leaf fragments were found in seven stomachs but were likely swallowed accidentally with prey items.

Prey volume in the dry season was on average 76.55 ± 9.05 mm^3 (total volume of 4,822.54 mm^3) and 117.88 ± 12.11 mm^3 (total volume of 8,487.45 mm^3) in the rainy season. Analysis of variance (ANOVA) for measurements of prey types showed that

TABLE 1. Dietary composition of adult female *Ichthyophis bannanicus* in the Mekong Delta, Vietnam (*N* = 135 stomach contents). F, total frequency; %F, relative frequency; N, total abundance; %N, relative abundance; V, total volume (mm^3); %V, relative volume; RI, relative importance index [RI = (%F + %N + %V)/3].

Prey category	F	%F	N	%N	V	%V	RI
Achatinidae	55	32.93	59	33.15	4,141.61	31.12	32.40
Araneae	3	1.80	3	1.69	205.12	1.54	1.67
Coleoptera	1	0.60	1	0.56	96.61	0.73	0.63
Diplopoda	2	1.20	2	1.12	318.32	2.39	1.57
Formicidae	31	18.56	35	19.66	1,841.45	13.84	17.35
Insect larvae	2	1.20	2	1.12	145.17	1.09	1.14
Isoptera	8	4.79	8	4.49	363.32	2.73	4.00
Lumbricidae	46	27.54	47	26.40	3,725.17	27.99	27.31
Orthoptera	5	2.99	5	2.81	421.11	3.16	2.99
Ranidae	2	1.20	2	1.12	987.45	7.42	3.25
Scolopendromorpha	3	1.80	3	1.69	647.22	4.86	2.78
Unidentified	9	5.39	11	6.18	417.39	3.14	4.90

TABLE 2. Comparison of dietary breadth and occurrence of prey items in adult female *Ichthyophis bannanicus* in the Mekong Delta, Vietnam. (1) Lap Vo ($N = 22$), (2) Tam Nong ($N = 26$), and (3) Hong Ngu ($N = 21$) in Dong Thap Province; and (4) Thoai Son ($N = 20$), (5) Tri Ton ($N = 20$), and (6) Tinh Bien ($N = 26$) in An Giang Province.

	Dong Thap			An Giang			
	(1)	(2)	(3)	(4)	(5)	(6)	Total ($N = 135$)
Prey/diet breadth	3.41	4.01	4.48	3.79	4.27	5.96	4.52
Achatinidae	41.18	37.78	30.77	36.36	39.29	29.63	35.33
Araneae	–	2.22	–	–	3.57	3.70	1.80
Coleoptera	–	–	2.56	–	–	–	0.60
Diplopoda	–	2.22	–	–	–	3.70	1.20
Formicidae	17.65	20.00	28.21	18.18	17.86	18.52	20.96
Insect larvae	–	–	2.56	–	3.57	–	1.20
Isoptera	5.88	4.44	2.56	9.09	3.57	7.41	4.79
Lumbricidae	29.41	28.89	25.64	36.36	32.14	22.22	28.14
Orthoptera	5.88	2.22	2.56	–	–	7.41	2.99
Ranidae	–	–	2.56	–	–	3.70	1.20
Scolopendromorpha	–	2.22	2.56	–	–	3.70	1.80
Categories	5	8	9	4	6	9	11

the total volume of prey categories, prey length, and prey width between seasons were different (volume, $F_{1,133} = 7.14$, $P = 0.008$; length, $F_{1,133} = 5.22$, $P = 0.024$; and width, $F_{1,133} = 8.08$, $P = 0.005$), whereas the amount of prey types did not differ ($F_{1,133} = 3.05$, $P = 0.083$). An ANOVA on the total volume of prey categories between Dong Thap (8,726.60 mm^3) and An Giang (4,583.39 mm^3) Provinces indicated that prey volume, prey length, and number of prey, but not prey width, were different among localities (volume, $F_{1,133} = 14.35$, $P < 0.001$; length, $F_{1,133} = 17.46$, $P < 0.001$; number, $F_{1,133} = 7.49$, $P = 0.007$; and width, $F_{1,133} = 3.55$, $P = 0.062$). A two-factor analysis of covariance (site-season ANCOVA) on the total volume of prey types with total length as a covariate ($F_{1,130} = 24.61$, $P < 0.001$) and mouth width ($F_{1,130} = 35.472$, $P < 0.001$) was different. Also, we used a multivariate analysis of variance (two-factor MANOVA) to examine the effects of season and site factors on the volume of three dominant prey categories (Achatinidae, Formicidae, and Lumbricidae; see Fig. 5) for populations of the Bannan Caecilian showing the season and site factors or season–site interactions were significant (season: Wilks' $\lambda = 0.343$, $F_{3,22} = 14.031$, $P < 0.001$; site: Wilks' $\lambda = 0.897$, $F_{15,61} = 3.164$, $P = 0.001$; and season–site: Wilks' $\lambda = 0.281$, $F_{15,61} = 2.382$, $P = 0.009$).

Adult females of the Bannan Caecilian showed positive correlations between TL and MW and prey size consumed, with a correlation between TL and prey size (TL and prey length: $r = 0.519$, $P < 0.001$; TL and prey width: $r = 0.243$, $P = 0.004$; and TL and prey volume: $r = 0.477$, $P < 0.001$) and between MW and prey size (MW and prey length: $r = 0.622$, $P < 0.001$; MW and prey width: $r = 0.219$, $P = 0.011$ and MW and prey volume: $r = 0.522$, $P < 0.001$). There were strong positive correlations between the morphological measurements (TL and MW: $r = 0.873$, $P < 0.001$, Fig. 6A; MW and BM: $r = 0.872$, $P < 0.001$, Fig. 6B; and TL and BM: $r = 0.958$, $P < 0.001$, Fig. 6C). The results of multiple linear regressions between prey size (length, width, and volume) and body size (TL, BM, and MW) were positively significant (prey length: $r^2 = 0.396$, $F_{3,131} = 28.581$, $P < 0.001$, Fig. 7A; prey width: $r^2 = 0.061$, $F_{3,131} = 2.771$, $P = 0.044$, Fig. 7B; and prey volume: $r^2 = 0.276$, $F_{3,131} = 16.652$, $P < 0.001$, Fig. 7C).

Multiple regression results for possible effects of temperature and rainfall on prey volume were significant among seasons ($r^2 = 0.066$; $F_{2,132} = 4.689$, $P = 0.011$), whereas precipitation, but not temperature, was associated positively with prey volume (rainfall: $r^2 = 0.067$; $F_{1,133} = 9.432$, $P = 0.033$, $\beta = 0.262$; temperature: $r^2 = 0.012$; $F_{1,133} = 1.391$, $P = 0.241$, $\beta = 0.011$).

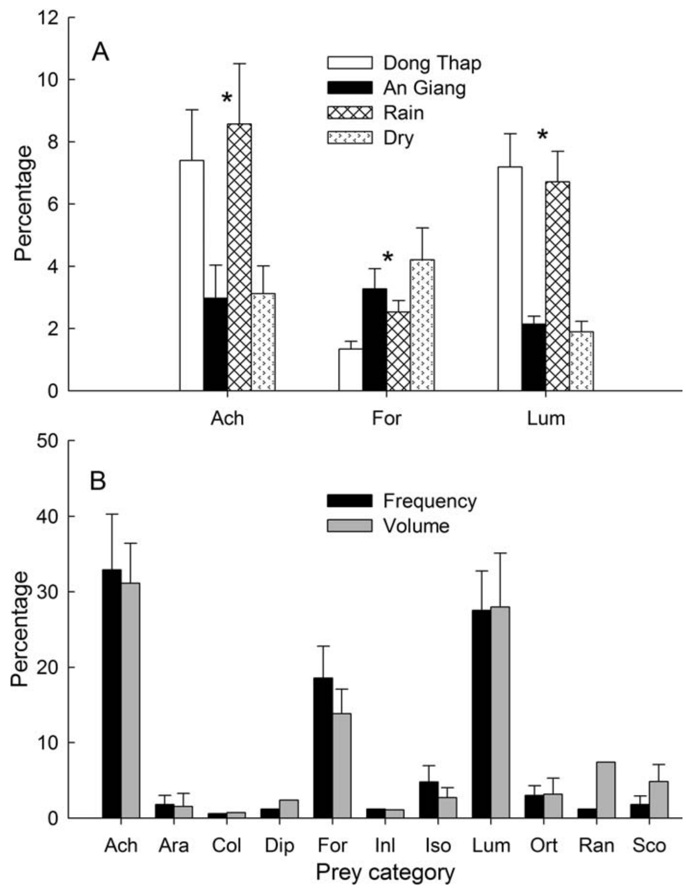

FIG. 5. Diet of *Ichthyophis bannanicus* based on total stomach contents ($N = 135$) in the Mekong Delta, Vietnam. (A) Mean percent volume of the most important prey categories (Ach, Achatinidae; Lum, Lumbricidae; For, Formicidae) and (B) frequency of occurrence and percent volume of each prey category identified in stomachs of the Bannan caecilians. Iso, Isoptera; Ran, Ranidae; Ort, Orthoptera; Sco, Scolopendromorpha; Dip, Diplopoda; Ara, Araneae; Inl, Insect Larvae; and Col, Coleoptera. Data are presented as mean ± SD, for each important prey category on shape A; the asterisks show where a significant among-site or -season difference occurred using the two-factor MANOVA analysis.

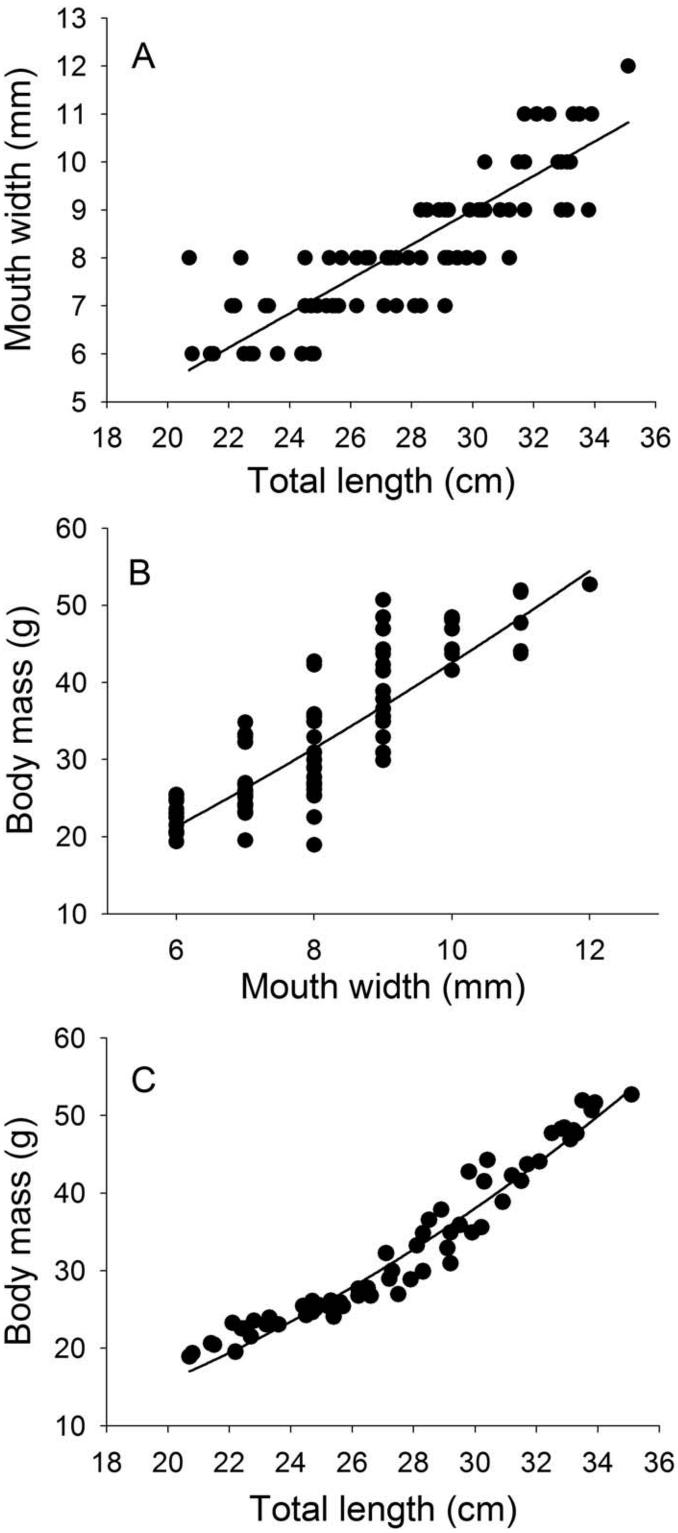

FIG. 6. Dispersion diagrams from Pearson's correlations between total length and mouth width (A), mouth width and body mass (B), and total length and body mass (C) of adult female *Ichthyophis bannanicus* in the Mekong Delta, Vietnam.

Results of multiple linear regressions for the effects of temperature and rainfall on prey number indicated no significant effect (overall: $r^2 = 0.0241$; $F_{2,132} = 1.621$, $P = 0.206$; temperature: $r^2 = 0.0112$; $F_{1,133} = 1.492$, $P = 0.224$; and rainfall: $r^2 = 0.0221$; $F_{1,133} = 2.911$, $P = 0.093$).

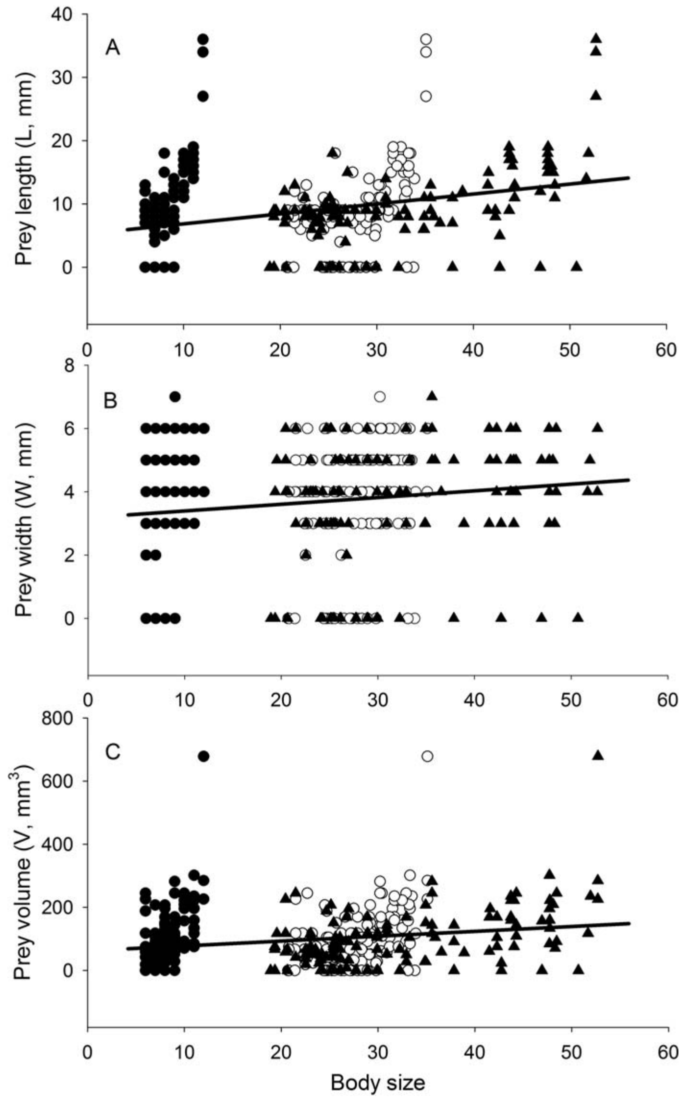

FIG. 7. Relationship between prey size (prey length [L, mm], prey width [W, mm], and prey volume [V, mm^3]) and body size (total length [TL, cm], open circles; mouth width [MW, mm], solid circles; and body mass [BM, g], solid triangles) of adult females of *Ichthyophis bannanicus* in the Mekong Delta, Vietnam. Linear regression equations are (A) prey length, $L = 2.73\text{MW} + 0.168\text{BM} - 0.536\text{TL} - 3.89$; (B) prey width, $W = 0.049\text{MW} - 0.015\text{BM} + 0.145\text{TL} - 0.28$; and (C) prey volume, $V = 25.2\text{MW} + 1.52\text{BM} + 0.145\text{TL} - 125$.

DISCUSSION

Previous studies indicate that for most amphibian species living in tropical regions, fewer animals are captured during the dry season compared to wet and rainy seasons (Wake, 1980; Savage and Wake, 2001; Measey and Di-Bernardo, 2003; Vyas, 2004; Kupfer et al., 2005a,b). In this study, we collected relatively equal proportions of caeclians in both dry ($N = 63$) and wet ($N = 72$) seasons. In the dry season, the number of *I. bannanicus* collected underground or under leaf litter was larger compared to above ground, whereas in the rainy season, most *I. bannanicus* were found above ground. Previous studies on feeding ecology and abundance of caecilians generally report data obtained during the rainy season (Oomen et al., 2000; Gaborieau and Measey, 2004; Measey et al., 2004; Kupfer et al., 2005a,b; Maciel et al., 2012).

Size dimorphism among populations have been reported for amphibian species, including anurans, caecilians, and newts

TABLE 3. Richness of potential prey available in the habitat of *Ichthyophis bannanicus* in Dong Thap and An Giang Provinces, the Mekong Delta, Vietnam, showing snails, ants, and earthworms were the most abundant prey in the study sites (Hoang, 2012). *Including Araneae, Coleoptera, Diplopoda, Ranidae, and Scolopendromorpha.

Prey	Dong Thap	An Giang
Achatinidae	35.20	18.90
Formicidae	17.69	37.08
Insect larvae	2.79	4.55
Isoptera	2.61	2.15
Lumbricidae	32.96	25.12
Orthoptera	3.91	4.55
Other categories*	4.84	7.66
Total	100	100

(Wells, 2007; Uzüm and Olgun, 2009; Amat et al., 2010; Hassine et al., 2011; Streicher et al., 2012). Similarly, we showed that adult female caecilians of *I. bannanicus* from three populations in Dong Thap Province (DT) have larger total length, mouth width, and higher body mass than animals from populations in An Giang Province (AG).

Thus far, we have not found male *I. bannanicus* in the Mekong Delta although we have made several attempts during the study period (12 months) by digging to a maximal depth of 45–55 cm, over an area of 100 m^2 (10 $\times$ 10 m) at each site/month where adult female caecilians of *I. bannanicus* were found or in the area where the clutch was found. To our knowledge, there is no published information about male caecilians of *I. bannanicus* being distributed in Vietnam. Whether only female *I. bannanicus* exist in the Vietnamese Southwest and whether ambient temperature affects sexual change of *I. bannanicus* living in this region are interesting questions.

In many amphibian species, there is a positive correlation between body size (mouth width, total length or snout–vent length) and prey size (Wells, 2007; Ortega et al., 2009; Quiroga et al., 2009; Caldart et al., 2012). Experimental studies on the Bannan Caecilian and other amphibians suggest that the size of the meal in ad libitum conditions is related to the amphibian's stomach capacity and that its urge to eat is largely conditioned by daily and annual rhythms (Larsen, 1992; Wells, 2007; Hoang, 2012). Populations of *I. bannanicus* in DT consumed prey types with larger size and volume than populations in AG, whereas the diet breadth of populations in AG was considerably broader than populations in DT. Variation among diets of populations may be attributable to the difference in the sizes of individuals as well as prey availability (Duellman and Trueb, 1986; Wells, 2007).

Previous studies show that amphibian species living in tropical and moderate regions mainly eat insects and some other invertebrates (Biavati et al., 2004; Wu et al., 2005; Mahan and Johnson, 2007; Leavitt and Fitzgerald, 2009; Hothem et al., 2009). We identified 11 prey categories in stomachs of *I. bannanicus* (excluding unidentified insects). Snails (Achatinidae), ants (Formicidae), and earthworms (Lumbricidae) were the most important prey categories (>77% of the important quotient) for the populations that we studied. In comparison, the diet of terrestrial *I. kohtaoensis* from the Isan region of northeastern Thailand consisted mainly of three major prey groups: termites, ants, and megascolecid earthworms (Kupfer et al., 2005b). Both *I. bannanicus* and *I. kohtaoensis* (collected from the Mekong valley, Ubon Ratchathani Province, Thailand) consumed vertebrate prey such as ranid frogs (this study) and microhylid frogs (Kupfer et al., 2005b). In other regions and caecilian taxa, results vary. In a study on the diet of *Caecilia gracilis* (Gymnophiona: Caeciliidae) from the Brazilian Cerrado, caecilians consumed earthworms in fragments (64.4%) and some prey items belonging to Orthoptera and root fragments of plant ($N = 59$, Maciel et al., 2012). Maciel et al. (2012) found that 31% of the stomachs assessed were empty. The specialization on snails, earthworms, and ants in populations of *I. bannanicus* could be a response to natural fluctuations in prey populations. Previous studies on the diet of amphibians indicated that their food depends on abundance or natural fluctuations in prey populations (Duellman and Trueb, 1986; Simon and Toft, 1991; Zug, 1993; Wells, 2007). In our study area, DT and AG are isolated by the Tien and Hau Rivers (Fig. 1), abundance of snails and earthworms were higher in DT than AG, whereas abundance of ants was higher in AG than DT (Table 3). In fact, the Bannan Caecilians in the plain regions of DT consumed a significantly larger number of snails and earthworms compared to the mountain regions of AG (Fig. 5). Ants are the most conspicuous element of the leaf-litter arthropod fauna in the secondary tropical forests (Le, 2007; Hoang, 2012), being more diverse and abundant in the mountain regions of AG (Hoang, 2012). We suggest that snails and earthworms are energetically rewarding prey categories, being less sclerotized than ants (Redford and Dorea, 1984) and having high levels of carbohydrates (Marconi et al., 2002; Wells, 2007). Their great abundance and diversity in the plain regions of DT may favor their consumption by Bannan Caecilians, thus explaining why the populations of *I. bannanicus* in DT have larger body sizes compared to caecilians in AG.

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