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Diet of Introduced Bullfrogs (*Rana catesbeiana*): Predation on and Diet Overlap with Native Frogs on Daishan Island, China

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ABSTRACT.—**We examined diet of introduced Bullfrogs (*Rana catesbeiana*) and three native frog species (*Rana limnocharis*, *Rana nigromaculata*, and *Bufo bufo gargarizans*) co-occurring at a group of ponds on Daishan Island, east of China, to gain insight into the nature of potential interactions between Bullfrogs and native frog species. For postmetamorphic Bullfrogs, aquatic prey items dominated volumetrically. Prey size, diet volume and volumetric percentage of native frogs in diet increased with Bullfrog body size. The number and volumetric percentage of native frogs in the diet were not different for female and male Bullfrogs, and both were higher for adults than for juveniles. Diet overlap between males and juveniles was higher than that between males and females and between females and juveniles. Diet overlap with each native frog species of male Bullfrogs was lower than that of female Bullfrogs and juvenile Bullfrogs. We did not exam effects of Bullfrogs on native frogs but our results suggest that the primary threat posed by juvenile Bullfrogs to native frogs on Daishan Island is competition for food, whereas the primary threat posed by male Bullfrogs is direct predation. Female Bullfrogs may threaten native frogs by both competition and predation. These differences among Bullfrog groups may be attributed to differences in body size and microhabitat use.**

The Bullfrog (*Rana catesbeiana*) is listed as one of the 100 worst invasive alien species in the world (IUCN, 2003). Native to eastern North America, the Bullfrog has been widely introduced to many countries and appears to have caused the decline or extinction of some native amphibians (Moyle, 1973; Bury and Luckenbach, 1976; Fisher and Shaffer, 1996; Kiesecker and Blaustein, 1997; Kupferberg, 1997). Bullfrogs affect native frogs through direct predation and interference competition (Kiesecker and Blaustein, 1998; Pearl et al., 2004). They are generalist predators and appear to eat anything that fits into their mouth (Bury and Whelan, 1984). Their diet consists mainly of insects and crustaceans, often overlapping with that of the native frogs. Tadpoles of Bullfrogs may compete with native frogs for cover and food (Kupferberg, 1997; Kiesecker and Blaustein 1998; Lawler et al., 1999; Kiesecker et al., 2001; Boone et al., 2004).

The Bullfrog was introduced into Chinese Taiwan in 1924 and into mainland China in 1959. It has been widely bred as a food item in many provinces of China since the 1980s (Li and Xie, 2004). Some Bullfrogs have escaped from rearing pens or in the process of transportation and trade (Li and Xie, 2002; Wu et al., 2004). Naturalized populations of Bullfrogs have recently been found in some provinces (Wang and Xie, 2004; Wu et al., 2004). It is thought that the Bullfrog was one factor leading to the extinction of *Cynops wolterstorffi* in Lake Dian, Yunnan province (He, 1998) and to the population decline of *Paa liui* in Lugu Lake, Sichuan province (Li and Xie, 2004), but direct evidence is lacking. China has 321 amphibian species (Wang and Xie, 2004), one of the highest amphibian species counts in the world (WCMC, 1992). Thus, the potential threat of the Bullfrog to China's amphibian diversity may be great. We investigated the diet composition of postmetamorphic Bullfrogs on the Daishan Island, Zhejiang province, China.

MATERIALS AND METHODS

The study was conducted on Daishan Island (30°14′–30°20′N and 122°05′–122°14′E), Zhoushan archipelago (29°31′–31°04′N and 121°30′–123°25′E), east of mainland China. The Zhoushan archipelago was formed during the late Pleistocene (Wang and Wang, 1980). It was originally a part of mainland China, separated from the mainland about 9000 years ago. The topography, climate, vegetation, and fauna of the archipelago are similar to those of the mainland (Yiming et al., 1998). Daishan is the second largest island in Zhoushan archipelago, about 100 km^2 in size (Zhoushan City Government, 1992). The highest elevation is 257 m. The topography of Daishan consists of plains (40% of the total area) and hills (60% of the total area). It is in the subtropical monsoon climate zone and is highly seasonal. Spring is warm and rainy, beginning in late March and ending in mid-June; the summer is rainy and hot from mid-June to late September; autumn is dry from late September to late November. January is the coldest month (average temperature 5.0°C) and August is the hottest month (average temperature 27.3°C). The island is covered with subtropical evergreen broad-leaved forest. There are eight native frog species on the island (Gu and Jin, 1985; Yiming et al., 1998): *Rana limnocharis*, *Rana nigromaculata*, *Rana japonica*, *Rana plancyi*, *Rana latouchii*, *Bufo bufo gargarizans*, *Microhyla ornata*, and *Hyla chinensis*.

Bullfrogs now have reproducing populations throughout most of the island. The island was invaded by escapees from three Bullfrog farms. Two of the farms ceased operations in 1994, and the third closed in 2000. Our study focused on an area (30°18′15″N, 122°08′51″E) composed of 14 ponds connected by ditches. The study

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area was selected because it was accessible to investigators and Bullfrogs could be caught easily. It is about 800 m from one of the Bullfrog farms that closed in 1994. The study area was approximately 500 m long and 100 m wide. It contained ponds ranging from 50–1038 m^2 and from 0.8–2 m deep. Most ponds had extensive areas of submersed vegetation, primarily composed of water caltrops (*Trapa natans L.*). The ponds are surrounded by agricultural fields. Besides Bullfrogs, *Rana limnocharis*, *R. nigromaculata*, and *Bufo bufo gargarizans* are commonly found in and around the ponds.

We collected postmetamorphic Bullfrogs and native frogs in mid-May, July, and September 2004, in each month for one week. Frogs were captured by hand or dip net at night (1900–2130 h) with the help of a 12-volt DC lamp. We captured frogs in the water and on the bank within 2 m from the water edge (Clarkson and DeVos, 1986) along the shoreline trail of ponds. The microhabitat of each frog was classified as either pond (captured in the water) or bank (captured on the bank). All captured frogs were labeled with a tag on their toes according to the microhabitat, placed in separate plastic bags with holes, and taken indoors for further analysis. We measured snout–vent length (SVL; to the nearest 0.02 mm) and body mass (to the nearest 0.1 g) of each frog, and we identified the gender of Bullfrogs. Males were identified based on secondary sexual characteristics (e.g., nuptial pads and yellow pigment on the throat and chest). Individuals with SVL $<$ 92.00 mm (minimum size of male Bullfrog) were considered juveniles; others were considered females if they lacked male characteristics. The stomach contents of each frog was flushed using a 50 mL syringe, with a 120 mm long, 3 mm diameter plastic tube, within 4 h of capture (Measey, 1998). A small nipper with plastic on the end was used to gently open the frogs' mouth, and the plastic tube was inserted carefully into the mouth and then into the stomach where 50 mL of dechlorinated water was slowly injected. If no items were flushed from the stomach, the procedure was repeated twice. Stomach contents flushed out were captured in a plastic container and immediately filtrated with gauze. They were then preserved in 75% alcohol. Stomach contents were spread in a Petri dish, and all prey items were identified to the lowest possible taxon (usually family) and life stage with the aid of a magnifier (8×). The length and width of each prey item was measured with a caliper (to the nearest 0.02 mm). Frogs were released at the site where they were captured the next morning.

Data Analysis.—The volume of each prey item was calculated using the formula for an ellipsoid (see Magnusson et al., 2003):

$$\text{Prey volume} = 4/3\ \pi\ (\text{length}/2)(\text{Width}/2)^2$$

Volume was not calculated for plant and mineral material, and these items were not included in the diet analysis. The frogs with empty stomachs were not included in the diet analysis. We divided Bullfrogs into three groups: males, females, and juveniles. We classified each prey item as either terrestrial or aquatic prey depending on its primary habitat. Prey items that occurred in both aquatic and terrestrial habitats were classified as ambiguous (see also Werner et al., 1995).

We used a Simplified Morisita Index, one of the most commonly used measures of niche overlap (Krebs, 1999), to calculate the diet overlap between Bullfrog groups and between each Bullfrog group and native frog species.

$$C_H = \frac{2\sum_i^n p_{ij}p_{ik}}{\sum_i^n p_{ij}^2 + p_{ik}^2}$$

where C_H = simplified Morisita index of overlap between species j and species k; p_{ij} = proportion that resource i is of the total resources used by species j; p_{ik} = proportion that resource i is of the total resources used by species k; n = total number of resource types ($i = 1,2,3,\cdots,n$).

We used SVL as frog body size and prey width as prey size. All Bullfrogs (121 individuals) that contained at least one prey item in their stomach were ranked from the smallest to largest size. We divided this ranking into 11 equal samples and calculated the mean size for each sample. A Pearson regression was used to examine the relationship between the volumetric percentages of native frogs in the stomach of each sample (arcsine-transformed) and mean size of the Bullfrogs (ln-transformed). Pearson regressions were also used to examine the relationships between mean prey size of each Bullfrog (ln-transformed) and its size (ln-transformed) and between the diet volume of each Bullfrog (ln-transformed) and its size (ln-transformed).

We used Kruskal-Wallis ANOVA to examine differences among Bullfrog groups in numbers of native frogs consumed, volumetric percentage of native frogs in the stomach, mean prey size and body size. If one of these differences was significant, we used Mann-Whitney *U*-tests to do pairwise comparison of the corresponding variable among groups. Chi-square tests were used to compare the frequency of captures in the two microhabitat types among Bullfrog groups and native frog species; in cases with low sample size, Fisher's Exact test was used. Because both microhabitat and body size may affect the diet of frogs, we calculated mean SVL of each Bullfrog group and native frog species for both pond and bank microhabitats. We used Student's *t*-tests to examine the difference in mean body size between the two microhabitat types. All statistical analyses were performed using the SPSS statistical package (SPSS Base 7.5 for Windows User's Guide, Cary, SPSS Inc., NC, 1997). Results of statistical tests were considered significant at the conventional $P \leq 0.05$ (two tailed).

Results

Postmetamorphic Bullfrogs and native frogs belonging to three species (*R. limnocharis*, *R. nigromaculata* and *B. b. gargarizans*) captured on Daishan Island are listed in Table 1. We also captured three other native frog species, including *Rana japonica* (four frogs), *Rana latouchii* (two frogs) and *M. ornata* (two frogs) but excluded them from the analysis because of small sample sizes.

Arthropods, molluscks, annelids, and chordates were found in the stomach of Bullfrogs, along with plant materials and minerals (Table 2). Arthropods included 12 orders in four classes (Crustacea, Arachnoida, Insecta, and Myriapoda); the molluscks included two orders of Gastropoda; and annelids included two

TABLE 1. Frogs captured on Daishan Island. In the Total column, the number of frogs with stomach containing prey is in parentheses. Only frogs with stomach containing prey were included in the means and standard deviations.

Group/Species	15–21 May	14–20 May	16–22 September	Total	SVL Mean ± SD (mm)	Pond SVL Mean ± SD (mm) (Number)	Bank SVL Mean ± SD (mm) (Number)
Male Bullfrog	4	16	11	31 (28)	113.64 ± 13.86	113.88 ± 14.19 (25)	111.67 ± 13.05 (3)
Female Bullfrog	1	7	6	14 (13)	116.69 ± 22.06	125.33 ± 30.58 (6)	109.29 ± 7.45 (7)
Juvenile Bullfrog	32	33	22	87 (80)	70.98 ± 11.68	70.05 ± 10.81 (43)	67.41 ± 11.79 (37)
Rana nigromaculata	2	9	23	34 (30)	64.93 ± 11.35	71.00 ± 12.33 (4)	64.00 ± 11.16 (26)
Rana limnocharis	41	31	55	127 (95)	46.47 ± 4.72	43.55 ± 5.11 (31)	47.89 ± 3.81 (64)
Bufo bufo gargarizans	30	25	22	77 (74)	69.91 ± 10.45	70.30 ± 11.29 (37)	69.51 ± 9.67 (37)

classes (Oligochaeta and Hirudinea) with each class including one order. Chordata in the stomachs of Bullfrogs included three *R. limnocharis*, three *B. b. gargarizans*, two *R. nigromaculata*, one *R. japonica*, one bird (*Passer montanus*), one fish (*Misgurnus anguillicadatus*), and one tail of an unidentified lizard. Aquatic prey composed 50.76% (volumetric percentage) of the diet of female Bullfrogs, 63.02% of the male diet, and 54% of the juvenile diet (Table 2).

In the stomachs of males, Cambaridae (crayfish: *Procambarus clarkii*) was the most important prey item (52.9%), followed by Passeriformes (14.61%) and Raniformes (12.05%). However, for females the three most important items were Basommatophore (32.43%), Raniformes (24.43%), and Cassididae (16.75%). The three most important diet items for juveniles were Cambaridae (33.60%), Basommatophore (12.57%), and Odonata (11.35%).

There was a high degree of diet overlap between males and juveniles, but diet overlap between males and females and between females and juveniles was low (Table 3). Diet overlap with each native frog species was lower for male than for female and juvenile Bullfrogs.

Eight Bullfrogs, including four females, three males and one juvenile, preyed on native frogs. Predation on native frogs differed among Bullfrog groups (χ^2 = 16.637, df = 2, $P < 0.001$). Stomachs of males and females contained a similar number of native frogs (z = 0.117, P = 0.311), but the stomachs of both sexes contained more natives than did juvenile stomachs (for female, z = 4.353, $P < 0.001$; for male, z = 2.272, P = 0.023). The volumetric percentage of native frogs in the diet increased with the size of Bullfrog samples (Fig. 1), and the percentage was different among Bullfrog groups (χ^2 = 17.11, df = 2, $P < 0.001$). The volumetric percentage of native frogs in the diet did not differ between males and females (Mann-Whitney U-test, z = 1.731, P = 0.084) but was higher than juveniles for both females (z = 4.379, $P < 0.001$) and males (z = 2.239, P = 0.025).

Mean prey size for Bullfrogs was positively related to Bullfrog SVL (both were ln-transformed; Fig. 2). Prey size differed among Bullfrog groups and native frog species (χ^2 = 255.145, df = 5, $P < 0.001$). Prey size of male Bullfrogs (mean ± SD: 23.71 ± 18.29 mm) was the largest and was significantly larger than that of female Bullfrogs (18.11 ± 14.26 mm; z = 2.299, P = 0.022) and juvenile Bullfrogs (13.22 ± 11.49 mm; z = 5.010, $P <$ 0.001). Prey size of female Bullfrogs was larger than that of juvenile Bullfrogs (z = 2.584, P = 0.01). The total volume of prey items in the stomachs increased with Bullfrog body size (Fig. 3). Prey size of *R. nigromaculata* (14.27 ± 9.43 mm) was smaller than that of male Bullfrogs (z = 3.282, P = 0.001) and similar to that of female Bullfrogs (z = 1.370, P = 0.171) but larger than juvenile Bullfrogs (z = 2.102, P = 0.036). Both prey sizes of *R. limnocharis* (8.72 ± 8.43 mm) and *B. b. gargarizans* (8.24 ± 5.44 mm) were smaller than any Bullfrog group ($P < 0.01$ for all).

Body size differed among Bullfrog groups and native frog species (χ^2 = 282.283, df = 5, $P < 0.001$; Table 1). Mean size of male Bullfrogs was similar to that of female Bullfrogs (z = 0.417, P = 0.677), but both male and female Bullfrogs were larger than juvenile Bullfrogs and all of the native species ($P < 0.01$ for all). Juvenile Bullfrogs were larger than *R. nigromaculata* (z = 2.503, P = 0.012) and *R. limnocharis* (z = 11.768, $P < 0.001$), but no difference in mean size was found between juvenile Bullfrogs and *B. b. gargarizans* (z = 0.871, P = 0.384).

The relative frequency of captures in the two microhabitat types differed among Bullfrog groups and native frog species (χ^2 = 43.291, df = 5, $P < 0.001$; Table 1). More male Bullfrogs were captured in the ponds than female Bullfrogs (low sample size, Fisher's Exact Test, P = 0.005), juvenile Bullfrogs (χ^2 = 11.231, df = 1, P = 0.001), and all native frog species ($P < 0.001$ for all). The relative frequency of captures in the two microhabitat types for female Bullfrogs was similar to that for juvenile Bullfrogs (χ^2 = 0.259, df = 1, P = 0.611), *R. limnocharis* (low sample size, Fisher's Exact Test, P = 0.362), and *B. b. gargarizans* (χ^2 = 0.065, df = 1, P = 0.798) but different from that for *R. nigromaculata* (low sample size, Fisher's Exact Test, P = 0.044), which were more common on the banks. The relative frequency of captures in the two microhabitat types for juvenile Bullfrogs was similar to that of *B. b. gargarizans* (χ^2 = 0.517, P = 0.472) but different from that of *R. limnocharis* (χ^2 = 9.348, df = 1, $P < 0.001$) and *R. nigromaculata* (χ^2 = 15.84, df = 1, $P < 0.001$). The latter two species were more likely to be found on banks than were juvenile Bullfrogs.

Mean size of juvenile Bullfrogs captured in the ponds was larger than for juveniles captured on banks (t = 2.628, df = 78, P = 0.01; Table 1). Size did not differ between microhabitats for male Bullfrogs, female Bullfrogs, *R. nigromaculata*, and *B. b. gargarizans*

TABLE 2. Diet volumetric percentage for Bullfrog groups and native frog species. Prey habitat types are listed in parentheses and are as follows: A = aquatic, T = terrestrial, ? = ambiguous. Volume was not calculated for plants and minerals but was labeled "+" to denote presence. Prey items with a volumetric percentage < 1% were incorporated together as Miscellaneous.

Prey taxon	Female Bullfrog	Male Bullfrog	Juvenile Bullfrog	*Rana nigromaculata*	*Rana limnocharis*	*Bufo bufo gargarizans*
Arthropoda						
Crustacea						
Decapoda						
Cambaridae (A)	12.02	52.90	33.6	0	0	0
Isopoda (T)	0.91	0.54	10.32	5.45	23.44	49.79
Arachnoida						
Araneida (T)	0.06	0.32	1.19	0.11	2.92	0.05
Insecta						
Diptera						
Larvas (A)	0.16	0	0	0	1.23	0.07
Coleoptera						
Carabidae (T)	0	0	0.20	2.00	0.43	0.37
Cicindelidae (T)	0.70	1.17	4.39	0	0	0.57
Cassididae (T)	16.75	0	0	0	0	0.06
Dytiscidae (A)	6.15	2.23	0.20	0	0	0.12
Tenebrionidae (T)	0.11	0.13	1.54	2.01	2.43	1.03
Cerambycidae (T)	0	0	0	1.79	0	0
Lampyridae (T)	0	0	1.17	0.83	0.07	1.72
Orthoptera						
Acridiidae (T)	3.48	0.05	0.33	6.91	4.33	0
Cryllotalpidae (T)	0	0.77	3.27	0.53	3.82	0
Tetrigidae (T)	0	0.34	0	2.70	0	0
Cryllidae (T)	0	0	0	0.13	2.45	0.03
Lepidoptera						
Larvas (T)	1.19	1.17	1.67	6.75	11.96	1.92
Hemiptera						
Belostomatidae (A)	0	0	2.59	0	0	0
Coreidae (T)	0	0	0.13	1.14	0	0.56
Pentatomidae (T)	0	0	0.95	2.19	1.15	0.88
Diplura						
Japygidae (T)	0	0.06	0	0	0	1.64
Dermaptera (T)	0	0	0.07	0.03	1.59	0.22
Odonata (T)	0	5.10	11.35	0.31	8.43	0
Myriapoda						
Scolopendromorpha (T)	0	0.04	0.58	0	2.41	0.81
Mollusca						
Gastropoda						
Basommatophore (A)	32.43	7.83	12.57	24.90	3.48	37.16
Stylommatophore (T)	1.41	0	1.37	30.90	0	0
Annelida						
Oligochaeta						
Ophisthopora (T)	0	0	1.25	0	22.86	0
Hirudinea						
Rhynchobdellida (A)	0	0.06	3.14	0	0	0.32
Chordata						
Amphibia						
Raniformes (?)	24.43	12.05	3.76	10.50	2.88	0
Pisces						
Cypriniformes (A)	0	0	1.90	0	0	0
Aves						
Passeriformes (T)	0	14.61	0	0	0	0
Miscellaneous	0.21	0.64	2.47	0.79	4.14	2.68
Plant	+	+	+	+	+	+
Mineral	+	+	+	+	+	+
Aquatic prey	50.76	63.02	54.00	24.90	4.71	37.67
Terrestrial prey	24.61	24.30	39.78	63.78	88.29	59.65

TABLE 3. Diet overlap index between Bullfrog groups and native frog species.

	Female Bullfrog	Male Bullfrog	Juvenile Bullfrog
Male Bullfrog	0.4463		
Juvenile Bullfrog	0.4944	0.8220	
Rana nigromaculata	0.5776	0.1321	0.2782
Rana limnocharis	0.1338	0.0598	0.3171
Bufo bufo gargarizans	0.4178	0.0905	0.3644

($P \geq 0.258$ for all). Mean size of *R. limnocharis* captured in the pond was smaller than for those captured on bank ($t = 4.641$, df = 93, $P < 0.001$).

DISCUSSION

The diet of Bullfrogs on Daishan Island, China, is similar to that of Bullfrogs from other areas (e.g., Clarkson and DeVos, 1986; Werner et al., 1995; Hirai, 2004). First, similar to the findings of Werner et al. (1995) and Hirai (2004), the aquatic prey items were the most important in terms of volume. Among aquatic prey, crayfish were one of the most important species (Clarkson and DeVos, 1986; Hirai, 2004). Second, anurans comprised a large portion of the total prey volume. Many studies demonstrated that other frogs, particularly ranids, were very important components of Bullfrog's diets (for review, see Werner et al., 1995). Third, the prey size and diet volume of Bullfrogs increased with their size, which is consistent with gape limitation (Werner et al., 1995).

Microhabitat and body size were both associated with differences in diet among Bullfrog groups. Using the percent overlap index, Werner et al. (1995) reported that the diet overlap between male Bullfrogs and female Bullfrogs was higher than that between adult and juvenile Bullfrogs. Contrary to this finding, we found lower diet overlap between male and female Bullfrogs than between adult and juvenile Bullfrogs. This difference may be attributed to differential microhabitat use of males and females in our study.

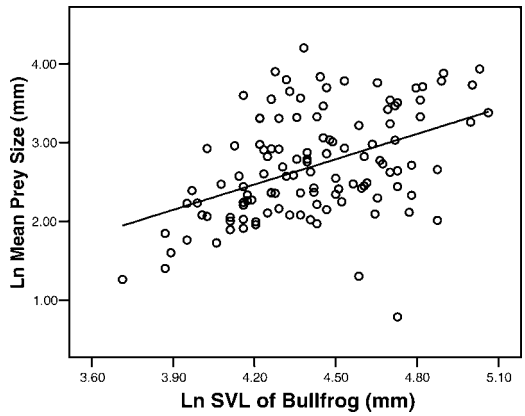

FIG. 2. Mean prey size as a function of Bullfrog SVL (snout–vent length; $r = 0.4503$; df = 119; $P < 0.01$).

In Werner et al.'s (1995) study, almost all of the male and female Bullfrogs were captured in ponds as opposed to on bank and presumably had similar prey availability. In our study, males were more likely to be found in the ponds compared to on bank than were the females. Consistent with this microhabitat difference, females seemed to ingest more Basommatophore, which are common along the bank while males had more crayfish in their diet.

Male Bullfrogs had a higher diet overlap with juvenile Bullfrogs than did female Bullfrogs. Male Bullfrogs and juvenile Bullfrogs both had a higher volumetric percentage of aquatic food items in their diet than did females, which may explain the higher diet overlap between male Bullfrogs and juvenile Bullfrogs. For both male and juvenile Bullfrogs, crayfish was the most important prey item in their diet in terms of volume.

The positive correlation between volumetric percentage of native frogs in Bullfrog diets and body size of Bullfrogs indicated that larger Bullfrogs preyed on

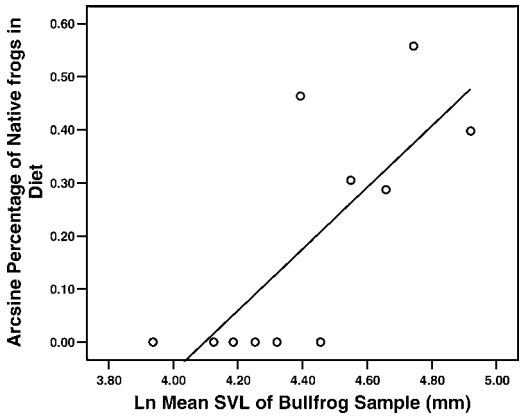

FIG. 1. Volumetric percentage of native frogs in the diet as a function of mean SVL (snout–vent length) of Bullfrog samples ($r = 0.7595$; df = 9; $P < 0.01$).

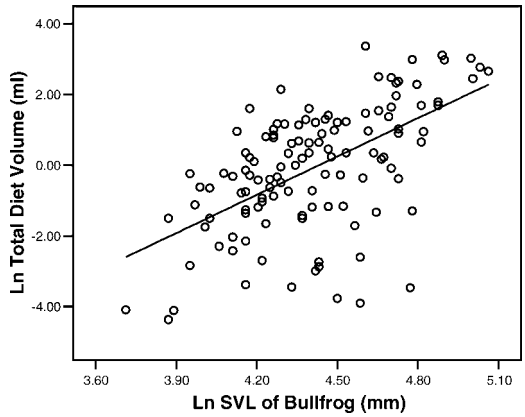

FIG. 3. Total diet volume as a function of Bullfrog SVL (snout–vent length; $r = 0.5737$; df = 119; $P < 0.01$).

more native frogs than did smaller Bullfrogs. In fact, male and female Bullfrogs preyed on more native frogs than juvenile Bullfrogs, and volumetric percentage of native frogs in the diet of both sexes were higher than juvenile Bullfrogs. This is consistent with the general pattern of adults preying on other frogs more than juveniles (Werner et al., 1995) and is consistent with gape limitation. Vertebrate prey items, especially native frogs, tend to be the largest prey items that we found in Bullfrog stomachs. Many of them were approximately the same size as or larger than some of Bullfrogs. Smaller Bullfrogs, such as some juveniles, could not prey on native frogs. Male Bullfrogs and female Bullfrogs had similar body size and a similar volumetric percentage of native frogs in their diet but seemed to select different microhabitats, suggesting that both microhabitats had native frogs available as prey for Bullfrogs.

Diet overlap of native frog species with Bullfrogs depended on Bullfrog group and generally coincided with microhabitat preference: species or groups occupying similar microhabitats had similar diets. Werner et al. (1995) documented that body size was related to diet overlap. This pattern can be found in *R. limnocharis* in our study. Among the three native frog species that we examined, *R. limnocharis* had the lowest diet overlap and largest size difference compared to each Bullfrog group. The association between body size and diet overlap may be related to prey size (Werner et al., 1995). Smaller species generally fed on smaller food items.

Werner et al. (1995) reported different effects of adult and juvenile Bullfrogs on Green Frogs (*Rana clamitans*). Our results suggest a similar hypothesis that, because of differences in diet overlap and evidence of direct predation on natives, juvenile Bullfrogs may affect natives by competition, males may affect natives by predation, and females may affect natives by both mechanisms. The differences in diet among Bullfrog groups appear attributable to differences in body size and microhabitat selection.

The Bullfrog has been identified as a factor in the decline of native frog species in some regions where it has been introduced (for a review, see Pearl et al., 2004) and our study suggests the potential for Bullfrogs to be a threat to some species on Daishan Island. Island biotas have long been considered more vulnerable to the negative effects of invaders than are mainlands (Elton, 1958; Wilson, 1965), and introduced species have caused more extinction on islands than on mainlands (Atkinson, 1989). We urge further study and monitoring of Bullfrogs on both Daishan Island and mainland China to assess current and future impacts on the native fauna.

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Hind-Limb Length Plasticity in *Anolis carolinensis*

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ABSTRACT.—The positive relationship between hind-limb length and perch diameter is well established in *Anolis* lizards, both among populations of some species and among species. Interspecific comparisons indicate that longer legs confer an advantage for increased running speed on broad substrates, whereas shorter limbs provide greater maneuverability on narrow surfaces. In this light, phenotypic plasticity for hind-limb length previously detected in *Anolis sagrei* may be adaptive because hatchlings exposed to only broad substrates developed relatively longer hind limbs for their body size compared to hatchlings exposed to only narrow substrates. We tested the generality of hind-limb length plasticity in *Anolis* by conducting a hatchling growth experiment on *Anolis carolinensis*, a distant relative of *A. sagrei* and a different type of habitat specialist. Similar to *A. sagrei*, *A. carolinensis* grown in cages with different sized perches showed hind-limb length plasticity, but the magnitude of difference between treatments and sexes was less for *A. carolinensis* than for *A. sagrei*. This finding suggests either hind-limb plasticity is widespread within the genus *Anolis* or that it has evolved independently at least twice.

A recent study revealed hind-limb length plasticity in the lizard *Anolis sagrei* (Losos et al., 2000). Hatchling lizards reared with only broad perches had significantly longer hind limbs for a given body size than those raised with only narrow perches; this was the first study to show this type of plasticity in a vertebrate. Furthermore, this plasticity mirrored the correlation between hind-limb length and perch diameter found among natural (Losos et al., 1994) and experimental (Losos et al., 1997) populations of this species. That is, *A. sagrei* shows a positive relationship between the mean perch diameter and the mean relative hind limb length among populations. This same relationship, greatly magnified, also exists among species in the *Anolis* radiation (Williams, 1983; Losos, 1990). The functional significance of the relationship between hind-limb length and perch diameter is clear: species with relatively longer legs run faster on broad surfaces, whereas those with relatively shorter legs are better

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suited for maneuvering on narrow substrates (Losos and Sinervo, 1989; Losos and Irschick, 1996; Irschick and Losos, 1998; Spezzano and Jayne, 2004). Although intraspecific studies of the functional relationship between hind-limb length and perch diameter are lacking, similar biomechanical factors likely apply within species as well.

Although hind-limb plasticity has implications for adaptive differentiation within a species, its importance in the broader context of the *Anolis* radiation is unclear. In particular, *A. sagrei* has perhaps the greatest breadth of habitat use among all anole species (e.g., Schoener, 1968; Losos et al. 1994; Mattingly and Jayne, 2004); if plasticity were to have arisen in a single species as an adaptation to variable habitat use, *A. sagrei* would be the most likely candidate. Alternatively, such plasticity could characterize all anoles or even a more inclusive clade (cf. Erickson, 1997). Determining whether plasticity in hind-limb length is a unique property of *A. sagrei*, as opposed to being widespread within the genus, is an important first step toward assessing its importance in the adaptive radiation of *Anolis* lizards.

To examine the generality of phenotypic plasticity in hind-limb length within the *Anolis* radiation, we conducted hatchling growth experiments on *Anolis carolinensis*. Distantly related to *A. sagrei* (Nicholson et al., 2005), *A. carolinensis* is a trunk-crown habitat specialist, whereas *A. sagrei* is a trunk-ground habitat specialist (Williams, 1983). Yet, similar to *A. sagrei*, a positive relationship between perch diameter and relative hind-limb length exists among populations of *A. carolinensis* in the Bahamas (Losos et al., 1994). Thus, phenotypic plasticity may be important in hind-limb differentiation among populations of *A. carolinensis*. This experiment was designed to provide insight into the potential phylogenetic distribution of hind-limb plasticity as well as into its occurrence in other habitat specialists.

Materials and Methods

We conducted hatchling growth experiments from August 2002 through March 2003 and August 2003 through April 2004. Each year, 112 hatchling lizards were obtained from a commercial dealer in Laplace, Louisiana. Lizards were placed in plastic cages 28.5 × 17.5 × 21 cm, misted twice daily, and fed wingless fruit flies and hatchling crickets dusted with mineral supplements every two or three days. The bottoms of the cages were covered with traction sand and ZooMed Reptisun 5.0 UVB fluorescent bulbs provided lighting above each row of cages. Animal room conditions were maintained at 26°C, 70% relative humidity, and on a light:dark cycle of 13:11 h. The only perches available to lizards were either two narrow bamboo dowels 1 cm in diameter and 30 cm long ("narrow" treatment) or one piece of wood 23 cm long × 9 cm wide × 4 cm thick ("broad" treatment) leaned against the walls of the cages. In each year, 56 cages were used, with each cage initially containing two lizards. Lizards were haphazardly assigned to cages with the requirement that lizards placed in the same cage were matched for size, usually < 2 mm difference in snout–vent length (SVL), and then randomly assigned to a treatment. Cages were arranged on a metal rack with 14 per shelf in two rows and alternating treatments.

At the beginning of the experiment, prior to assigning lizards to treatments, we measured SVL and hind-limb length (HL) to the nearest 0.5 mm using a ruler. The same person (JJK) measured all lizards in this study. HL was measured as the distance from the insertion of the limb into the body wall to the distal tip of the claw on metatarsal IV. One individual per cage was toe-clipped for future identification. At the end of each experiment, SVL and HL were measured and sex determined.

To reject the possibility that differences in starting conditions or survival influenced HL at the end of the experiment, we tested for differences in initial HL between treatments, sex, year, survival, and the interaction between treatment and survival using analysis of covariance (ANCOVA) with initial SVL as a covariate. To make the results of this study directly comparable to the previous study of *A. sagrei* (Losos et al., 2000), we determined whether the experimental treatment affected hind-limb growth in two ways. First, we tested for a difference in HL at the end of the experiment using ANCOVA with final SVL as the covariate and treatment, sex, and year as the factors. Second, we determined the difference in relative hind-limb growth (RHG) between the treatments. RHG was measured as ([final HL − initial HL]/[final SVL − initial SVL]) for all lizards surviving for the entire experiment. We evaluated RHG using a factorial three-way analysis of variance (ANOVA) that included treatment, sex, and year as factors. SVL, HL, and RHG were ln-transformed prior to analyses. *A priori* predictions for larger HL and RHG in broad treatment lizards and males were tested with one-tailed *P*-values, all other tests were two-tailed (Losos et al., 2000). If both lizards in a cage survived the entire experiment, then the mean values for SVL, HL, and RHG were calculated and used in subsequent analyses.

Results

Survival was 86% (96/112) and 55% (62/112) in the first and second years, respectively. At the start of experiments, ANCOVA showed no difference in HL while controlling for SVL between treatments ($F_{1,101} = 0.01$, $P = 0.935$), sexes ($F_{1,101} = 0.71$, $P = 0.401$), survival ($F_{1,101} = 0.19$, $P = 0.666$), or the interaction between treatment and survival ($F_{1,101} = 0.21$, $P = 0.644$; Table 1); slopes were homogeneous for all factors (all $P > 0.30$). There was, however, a significant difference between years ($F_{1,101} = 14.07$, $P = 0.0003$): relative hind-limb lengths at the start of the experiments were slightly longer for hatchlings in 2002 as compared to 2003.

At the end of the experiments, SVL growth did not differ between treatments ($F_{1,150} = 1.17$, $P = 0.281$), and no interactions among treatment, sex, and year were significant (all $P > 0.05$); but lizards grew more in 2002 than in 2003 ($F_{1,150} = 97.64$, $P < 0.0001$), and males grew more than females ($F_{1,150} = 11.27$, $P = 0.001$; Table 1). Analyzing cage means for final HL revealed lizards in the broad treatment ($F_{1,65} = 7.21$, $P = 0.005$, one-tailed) and males ($F_{1,65} = 22.14$, $P < 0.0001$, one-tailed) had longer hind limbs for a given body size (Fig. 1), whereas the effect of year was almost significant ($F_{1,65} = 3.50$, $P = 0.068$). Slopes were homogeneous for all factors (all $P > 0.15$) in this ANCOVA. Using cage means for RHG, the three-way ANOVA indicated that hind-limb growth rate was higher for broad treatment lizards ($F_{1,96} = 2.89$, $P = 0.046$, one-tailed) and males

TABLE 1. Initial and final snout–vent lengths (SVL), hind-limb lengths (HL), and relative hind-limb growth (RHG) for *Anolis carolinensis* by year, sex, and treatment. Values are mean ± SE (range, sample size). SVL and HL values (mm) are from individuals and RHG is based on cage means. Sample sizes are 209 for initial values (15 individuals were not sexed), 158 for final values (survivors only), and 104 for RHG (cage means).

Year	Sex	Treatment	Initial SVL	Initial HL	Final SVL	Final HL	RHG
2002	Male	Broad	27.1 ± 0.22 (25.0–29.5, 25)	17.2 ± 0.22 (15.0–19.5, 25)	41.9 ± 0.66 (34.5–47.0, 24)	28.0 ± 0.55 (22.0–32.0, 24)	0.722 ± 0.014 (0.636–0.867, 15)
		Narrow	27.0 ± 0.37 (24.0–30.5, 23)	17.1 ± 0.25 (15.5–19.5, 23)	41.8 ± 0.66 (36.5–47.5, 20)	27.5 ± 0.43 (24.5–31.5, 20)	0.717 ± 0.019 (0.608–0.840, 15)
	Female	Broad	26.3 ± 0.22 (24.5–28.0, 27)	16.7 ± 0.18 (15.0–19.0, 27)	38.7 ± 0.42 (34.0–44.5, 25)	25.3 ± 0.32 (22.5–29.0, 25)	0.687 ± 0.012 (0.633–0.769, 13)
		Narrow	26.8 ± 0.18 (24.5–28.5, 28)	16.9 ± 0.17 (15.5–19.5, 28)	38.7 ± 0.38 (35.5–43.5, 27)	24.7 ± 0.29 (22.0–29.0, 27)	0.648 ± 0.019 (0.476–0.746, 13)
2003	Male	Broad	27.0 ± 0.26 (25.0–29.0, 18)	16.8 ± 0.17 (15.5–18.5, 18)	36.2 ± 0.85 (32.0–39.5, 9)	23.5 ± 0.66 (20.5–26.0, 9)	0.719 ± 0.018 (0.688–0.789, 5)
		Narrow	26.9 ± 0.27 (24.5–31.0, 22)	16.7 ± 0.18 (15.0–18.0, 22)	37.3 ± 0.63 (32.0–40.0, 11)	24.0 ± 0.45 (20.5–26.0, 11)	0.704 ± 0.030 (0.636–0.870, 7)
	Female	Broad	27.0 ± 0.20 (24.0–29.0, 36)	16.6 ± 0.14 (14.5–18.5, 36)	36.3 ± 0.3 (33.0–39.0, 20)	23.2 ± 0.24 (21.5–25.0, 20)	0.732 ± 0.029 (0.556–1.083, 19)
		Narrow	27.0 ± 0.26 (24.5–29.5, 30)	16.8 ± 0.17 (15.0–18.5, 30)	36.1 ± 0.40 (32.0–40.0, 22)	23.0 ± 0.30 (20.0–26.0, 22)	0.683 ± 0.018 (0.560–0.813, 17)

($F_{1,96}$ = 3.40, P = 0.034, one-tailed; Fig. 2), but there was no effect of year ($F_{1,96}$ = 0.81, P = 0.371) or any interactions (all $P > 0.15$; Table 1).

DISCUSSION

This study demonstrates that hind-limb plasticity is not a unique property of *A. sagrei*. Rather, it also exists in *A. carolinensis*, a species adapted to a different habitat than its distant relative, *A. sagrei*. Uncorrected sequence divergence of 23.7% for the mitochondrial gene ND2 suggests these species diverged roughly 18 million years ago (Macey et al., 1998; Nicholson et al., 2005). Thus, either hind-limb plasticity arose independently multiple times in *Anolis* or the common ancestor of *A. carolinensis* and *A. sagrei* had hind-limb plasticity. The latter explanation suggests widespread hind-limb plasticity within the genus.

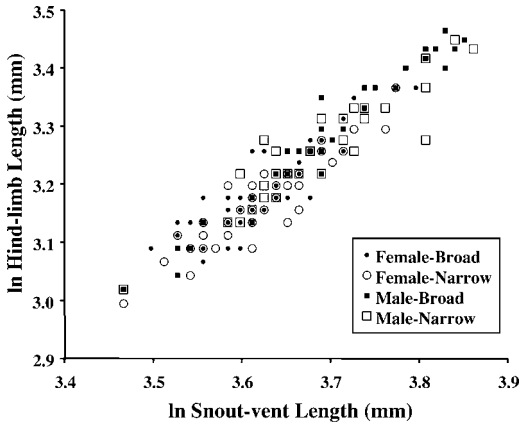

FIG. 1. The relationship between hind-limb length and snout–vent length for lizards in the two treatments at the end of the experiment. Data from the two years were pooled.

The pattern of hind-limb plasticity in *A. carolinensis* mirrors the positive relationship between hind-limb length and perch diameter observed among natural populations of this species (Losos et al., 1994). The 1 cm and 9 cm perch diameters used in this experiment are similar to perch diameters used by juvenile and subadult *A. carolinensis* in the field (range 0.3–10.8 cm; Schoener, 1968). Thus, the stimulus used to elicit the plastic response in hind-limb length in this experiment is biologically relevant to field conditions. The question still remains whether differences among populations are strictly genetically based, mediated through the environment (i.e., phenotypic plasticity), or a combination of both.

A parallel situation occurs in *A. sagrei*: a correlation exists among populations between hind-limb length

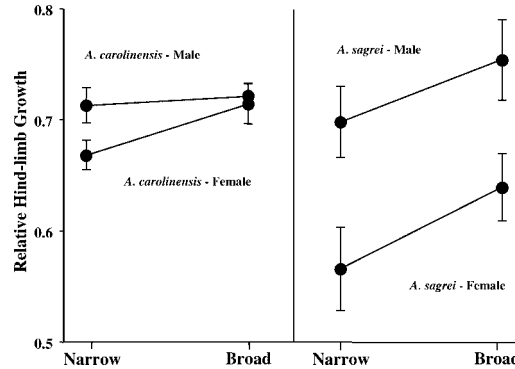

FIG. 2. Relative hind-limb growth (RHG) reaction norms for both sexes of *Anolis carolinensis* and *Anolis sagrei* using cage means. Values are means and error bars denote ± 1 SE. Data for *A. sagrei* are from Losos et al. (2000), but here raw RHG values are presented instead of ln-transformed values. See text for differences between the experiments that may complicate direct comparisons between the two species.

and habitat use (Losos et al., 1994), and laboratory studies reveal that hind-limb length is a plastic trait (Losos et al., 2000). Recent studies show natural selection on hind-limb length in Bahamian *A. sagrei* (Losos et al., 2004) and a high heritability for hind-limb length in a Florida *A. sagrei* population (J. Kolbe, L. Revell, and J. Losos, unpubl. data). Thus, natural selection on hind-limb length occurs, and a quantitative genetic basis for hind-limb variation exists in natural populations, which suggests that genetically based differences in hind-limb length might rapidly evolve among populations of *A. sagrei*. Similar studies in *A. carolinensis* may help clarify the cause of population differences in this species.

This study documents a second species of *Anolis* with phenotypic plasticity in hind-limb length. Broad substrates during hatchling development result in lizards with relatively longer hind limbs for both *A. sagrei* (Losos et al., 2000) and *A. carolinensis* (this study). Despite this similarity, several interesting differences in the pattern of hind-limb growth exist between the two species. Although there was a significant difference in hind-limb growth between males and females for both *A. carolinensis* and *A. sagrei*, the magnitude of the difference was much greater for *A. sagrei* (Fig. 2). Furthermore, the difference between male and female *A. carolinensis* was greater in the narrow treatment, whereas differences between male and female *A. sagrei* were similar in both treatments (Fig. 2). Thus, female *A. carolinensis* and both sexes of *A. sagrei* had similar, and much steeper, reaction norm slopes than male *A. carolinensis*.

Several caveats exist, however, to direct comparisons of reaction norms between these two species. First, some conditions varied between experiments with *A. carolinensis* and *A. sagrei*, including cage size, number of perches, and slight differences in perch diameter. Second, experiments with *A. carolinensis* lasted 2–3 months longer than those with *A. sagrei*. Thus, the magnitudes on RHG are not standardized for time. Finally, the two species grew over different size ranges. *Anolis carolinensis* grew on average from 26.9–38.7 mm SVL, whereas *A. sagrei* grew from 33.0–42.2 mm SVL. These differences may confound interpretations because the shape of the hind-limb growth curve likely varies at different times during development and among species (T. Sanger, pers. comm.).

Although it would be difficult, if not impossible, to fully reconstruct the role of phenotypic plasticity in the adaptive radiation of *Anolis*, the primary importance of interspecific competition in driving the evolutionary radiation of *Anolis* provides a good focal point for future work (reviewed in Losos, 1994). Many comparative (Jenssen, 1973; Schoener, 1975) and experimental (Pacala and Roughgarden, 1982; Rummel and Roughgarden, 1985) studies show anoles shift their structural habitat use in the presence or absence of congeners. These shifts likely lead to changes in perch diameter that could provide the stimulus for a plastic response in hind-limb length. Designing seminatural experiments using likely competitors and realistic perch characteristics will provide good tests of whether phenotypic plasticity in hind-limb length could have played a role in the *Anolis* radiation. Future studies should also standardize experimental conditions, duration, and growth range of lizards to facilitate direct comparisons of reaction norms among species as well as include more divergent habitat specialists, such as twig anoles.

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Peaked Temporal Pattern of Embryonic Metabolism in an Emydid Turtle (*Chrysemys picta picta*)

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ABSTRACT.—Temporal patterns of embryonic metabolism integrate ontogenetic changes in the energetic costs of growth and maintenance. Unlike most other chelonians, which show a peaked pattern of embryonic metabolic rate (MR) over time, turtles of the family Emydidae have been depicted with unimodally increasing patterns. We incubated eggs of the emydid *Chrysemys picta picta* under standard conditions at 30°C and serially measured rates of oxygen consumption. Five eggs showed clear decreases in MR before hatching; two others that hatched during MR measurements showed increases in MR associated with muscular activity. MRs of hatchling turtles were lower than those of prehatching embryos for at least 60 days, suggesting persistent costs of biosynthesis in yolk-dependent late-stage embryos and early hatchlings.

In oviparous tetrapods, temporal patterns in the metabolic rates of developing embryos reflect ontogenetic changes in the energetic costs of growth and maintenance, which are in turn functions of growth rate and body mass (Vleck et al., 1980; Hoyt, 1987; Vleck and Hoyt, 1991). Chelonians have been reported to exhibit several patterns of developmental metabolism. Early reports depicted a unimodal increase in metabolic rate over time for species from several families (Lynn and von Brand, 1945; Ackerman, 1981). However, sigmoidal and peaked patterns were reported subsequently (Gettinger et al., 1984; Webb et al., 1986; Thompson, 1989; Leshem et al., 1991). One review characterized the chelonian pattern as "logistic" (Packard and Packard, 1988), but Ewert (1991), citing unpublished data, opined that a peaked pattern "may typify most turtles." Indeed, with one exception (Thompson, 1993), the few subsequent studies on turtle eggs have all shown distinct declines from a peak, both in previously unstudied species (Booth 1998a,b, 2000) and in species previously reported as sigmoidal (Birchard and Rieber, 1995; Booth and Astill, 2001). The peaked pattern has been interpreted as reflective of a "resting" stage in which growth slows or stops before hatching (Webb et al., 1986; Thompson, 1989; Ewert, 1991), and this is commonly believed to facilitate synchronous hatching within nests (Thompson, 1989; Whitehead and Seymour, 1990).

The large, diverse family Emydidae has been poorly represented in studies of chelonian developmental metabolism. Lynn and von Brand (1945) fit a single sigmoidal curve to mixed data for three species, the emydids *Chrysemys picta picta* and *Terrapene carolina triunguis* and a kinosternid (*Kinosternon subrubrum*). Their individual-species data are sufficiently variable that they have been interpreted as both "logistic" (Packard and Packard, 1988:table 1) and "peaked" (Whitehead and Seymour, 1990:table 3); to our eyes, they do not clearly decline from a maximum. The only other emydid profiles in the literature are presented in otherwise unpublished figures in review articles:

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Congdon and Gibbons (1990:fig. 8.5) depicted data for *Trachemys scripta* that increase unimodally (albeit in a unique pattern), and Vleck and Hoyt (1991) showed data (credited to D. R. Hoyt and R. Albers) for *Pseudemys concinna* that increase sigmoidally (as classified by Whitehead and Seymour, 1990) to a more-or-less steady plateau (although their data for the trionychid *Apalone spinifera* clearly decline from a peak).

Is it true that turtles of the family Emydidae exhibit developmental patterns that differ from nearly all other chelonians? We report serial measurements of the metabolic rates of developing *C. p. picta* to address this question.

Materials and Methods

Two clutches of *C. p. picta* were collected from gravid females trapped in Lake Sylva on the College of New Jersey's campus in Ewing, New Jersey. Eggs were obtained on 13–14 July 2004 by inducing oviposition with oxytocin injections (Ewert and Legler, 1978). Each egg was weighed and individually marked. Seven eggs (four from one clutch, three from the other) were incubated at a target temperature of 30°C in a single plastic shoebox; following recommendations of Packard and Phillips (1994), eggs were half-buried in a medium composed of equal parts (by mass) of sterile, dried vermiculite and deionized water (water potential approx. –150 kPa). The incubation temperature was verified continuously by a thermocouple taped below the center rack of the incubator and by a Stowaway TidBit datalogger (Onset, Bourne, MA), inside the shoebox itself, which recorded temperature every 15 min (mean ± SD: T_{inc} = 29.83°C ± 0.40, N = 4505). Twice per week, the shoebox was weighed, water was replaced as needed, and the positions of eggs within the box and the box within the incubator were changed.

When an egg began pipping, a bottomless cup was placed around it to keep the hatchlings separate. Hatchlings were weighed, and each individual received a unique marking on the carapace. Hatchlings were kept in the box of vermiculite within the incubator until yolk sacs were completely absorbed. Thereafter, hatchlings were kept in plastic containers in 3 cm tap water and were exposed to laboratory room temperature (approximately 25°C), the natural daily photoperiod from a window, and timed 60-W incandescent bulbs (12:12 L:D) for basking. The hatchlings subsisted on yolk reserves after hatching and were offered commercial aquatic turtle food three times per week beginning at age 30 days.

The metabolic rates (MR) of developing eggs were measured as the rate of oxygen consumption at 30°C approximately once per week during the incubation period. The MR of hatchlings was also measured at 30°C at approximate ages 20, 70, and 160 days postpipping. Oxygen consumption was measured using closed-system respirometry (Vleck, 1987). Before each measurement of metabolic rate, hatchlings were fasted at least three days. Each egg or hatchling was weighed to the nearest 0.01 g, and randomly assigned to a 200-mL airtight plastic respirometry chamber, each equipped with two stopcocks. Before each set of measurements, three background air samples were obtained into 20-mL plastic syringes fitted with stopcocks. The chambers containing eggs or hatchlings were sealed, placed into the incubator, and after approximately one hour, chambers were removed from the incubators and 15-mL air samples were taken from stopcocks into syringes. Air from outside the building was pulled sequentially through a desiccant column, a mass flow controller (Sable Systems, Las Vegas, NV) that maintained a precise flowrate of 100 mL/min; a second, smaller (3 mL) desiccant column, and an oxygen analyzer (Sable Systems FC-1B) interfaced to a computer running Sable Systems DataCan acquisition software. Ten-milliliter subsamples of each air sample were injected into the airstream, and oxygen contents of dried background air samples and chamber samples were analyzed relative to dry, outside air with use of DataCan analysis software. Oxygen consumption was calculated according to Peterson (1990).

There was a possibility that MR would be increased above resting levels in hatchlings because they were able to move within the chambers. To increase the likelihood of obtaining a resting MR, each hatchling's MR was measured 3–4 times over a period of 2–3 days, and the minimum MR for each individual was selected for analysis.

Metabolic rates for the last five sampling periods (i.e., the last two egg measurements and the three hatchling measurements) were compared by repeated-measures ANOVA followed by posthoc pairwise comparisons (SYSTAT 11, Richmond, CA). Means are presented ± 1 SE.

Results

The mean incubation period (oviposition to pipping) at 30°C was 51 days (range 49–54 days). Eggs gained mass throughout incubation, increasing from 5.73 ± 0.12 to 6.14 ± 0.17 g. Hatchlings averaged 4.04 ± 0.12 g, 70.7% of initial egg mass.

Rates of oxygen consumption differed among sampling periods (rmANOVA, $F_{4,24}$ = 12.63, $P < 0.001$). Developing eggs exhibited a rapid increase in oxygen consumption between 14 and 30 days of development and a slower increase to day 42 (Fig. 1A). When the next measurements were made on day 50, four of the seven eggs had pipped. Two of these actually completed hatching in the respirometer during oxygen-consumption measurements, and they had slightly higher metabolic rates than previously (dotted circles in Fig. 1A). The other five eggs (including two that had pipped) had lower rates of oxygen consumption on day 50 than day 42 (Fig. 1A; posthoc pairwise comparison of repeated measures, P = 0.004). Declines ranged from 8–24% below maximum V_{O2}. Integration under the mean curve between oviposition and pipping (summation of areas of trapezoids, excluding the two hatching data) yielded a total of 349 mL O_2 consumed, equivalent to an energetic cost of 6.88 kJ (assuming 19.7 kJ/L O_2; Gettinger et al., 1984).

Hatchling turtles had uniformly lower metabolic rates at age 20 days (after pipping) than just before and during hatching (Fig. 1B; posthoc pairwise comparison of repeated measures, P = 0.012). Even 160 days after pipping, mean metabolic rate was similar to the maximum embryonic metabolic rate (Fig. 1B; posthoc pairwise comparison of repeated measures, P = 0.164), despite growth to 4.61 ± 0.23 g.

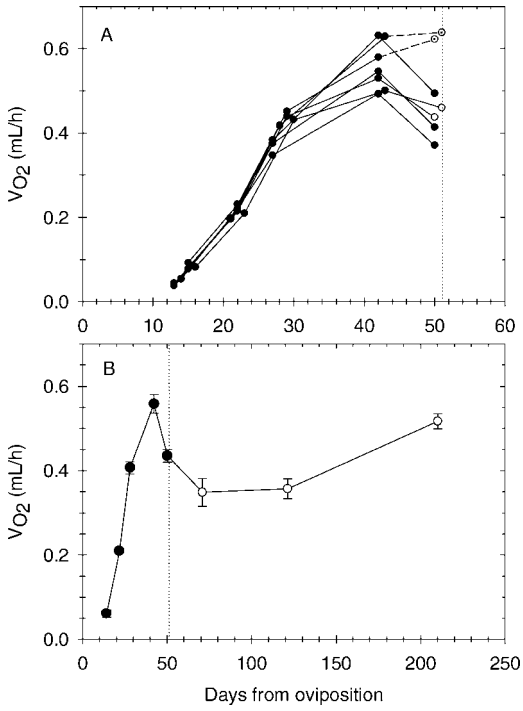

FIG. 1. Ontogeny of metabolic rates (oxygen consumption) of eggs and hatchlings of *Chrysemys picta picta*, measured at 30°C. (A) Sequential data for individuals ($N = 7$). Filled circles: eggs; open circles: pipped eggs; dotted circles: hatching hatchlings. (B) Mean values (± SE) for eggs (filled circles; same data as in A, omitting hatching data) and hatchlings (open circles). Vertical dotted line: mean pipping day.

DISCUSSION

The rate of oxygen consumption in eggs of *C. p. picta* clearly declined from a peak before hatching (Fig. 1). The only exceptions were two individuals that actually hatched during the measurement, and their slightly higher metabolic rate is attributable to muscular activity (cf. Lynn and von Brand, 1945). Two other individuals that had pipped but presumably remained motionless had metabolic rates typical of unpipped eggs of equal age (Fig. 1A), which corroborates Thompson (1989) and Whitehead and Seymour (1990).

Surprisingly, this is the first demonstration of a peaked developmental metabolic profile in the family Emydidae. Data published previously for *Chrysemys* (Lynn and von Brand, 1945), *Trachemys* (Congdon and Gibbons, 1990), and *Pseudemys* (Vleck and Hoyt, 1991) all seem to show different patterns. Peaked profiles are known for representatives of the families Chelydridae (Gettinger et al., 1984; Birchard and Rieber, 1995), Trionychidae (Gettinger et al., 1984; Vleck and Hoyt, 1991; Leshem et al., 1991), Carettochelyidae (Webb et al., 1986), Chelidae (Thompson, 1989; Booth, 1998a,b, 2000), Kinosternidae (Ewert, 1991), Bataguridae (Ewert, 1991; Leshem et al., 1991); Testudinidae (Leshem et al., 1991); and Cheloniidae (Booth and Astill, 2001). Thus, the pattern of growth that underlies the peaked metabolic profile seems firmly established as typical and, hypothetically, ancestral for chelonians.

Metabolic rate is an inherently integrative variable, and the metabolic profiles of developing and growing animals integrate energetic costs of maintenance and growth (Hoyt, 1987; Peterson et al., 1999). A peaked metabolic profile reflects a decrease in the rate and, hence, the costs of growth before hatching (Hoyt, 1987; Gettinger et al., 1984; Leshem et al., 1991; Booth, 1998a,b). In this context, the various temporal profiles are perhaps best viewed as a continuum in which the degree to which the metabolic rate asymptotes or declines at the end of the incubation period is caused by the degree to which growth slows before hatching. Such a view is supported by the observation that differences in embryonic growth rates, and therefore metabolic rates and temporal profiles, can also be caused by differences in water balance (Gettinger et al., 1984).

The resting metabolic rate of hatchling painted turtles continued to decline after hatching (Fig. 1B). The same phenomenon has been noted in other turtle species (Prange and Ackerman, 1974; Thompson, 1989; O'Steen and Janzen, 1999). It is likely that growth slows but does not completely stop, even in species with strongly peaked profiles (e.g., Leshem et al., 1991), such that late-stage embryos and very young hatchlings continue to pay the metabolic costs, similar to the biosynthetic component of SDA (Peterson et al., 1999), of converting residual yolk to body tissue (cf. Beaupre and Zaidan, 2001). If metabolic rates of late-stage embryos include a substantial cost of biosynthesis, then developmental costs of "maintenance" would be best measured some weeks after hatching, at a point when residual yolk is exhausted but growth fueled by ingestion has not yet begun (Steyermark and Spotila, 2000).

Adaptive explanations for the growth pattern that produces a peaked metabolic profile have centered on its putative utility in synchronizing hatching in nests containg embryos with different rates of development (Webb et al., 1986; Thompson, 1989, 1993; Whitehead and Seymour, 1990). However, an adaptation to ensure hatching synchrony seems wasted on painted turtles, which habitually overwinter within the nest and dig out the following spring (references in Ernst et al., 1994).

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