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THE HERPETOLOGICAL BULLETIN

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Front cover illustration. Madagascan tree boa Sanzinia madagascariensis © A. Radovanovic. See article on page 30.

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Feeding ecology and sexual dimorphism of *Enyalius perditus* in an Atlantic forest, Brazil

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ABSTRACT - We assessed the ecology, morphology and diet of *Enyalius perditus*. The data were compared among and within gender and collection periods. The specimens were collected in an anthropized biological reserve in the Atlantic forest biome, Brazil. The stomach contents were analyzed for number of prey, frequency, mass and volume. Sexual dimorphism occurred. No positive correlation between lizard size and volume of prey, nor between diet and collection period was found. *Enyalius perditus* diet was diverse comprising predominantly Formicidae, Orthoptera, Isoptera and other insect prey. In an urban forest, *E. perditus* was an opportunist predator of terrestrial arthropods. Differences in diet across gender were not found. Intersexual trophic similarities suggest there is no food resource partitioning in the population's diet studied herein.

C EXUAL dimorphism is a phenotypic difference D between males and females of the same species. Examples include differences in morphology, ornamentation and behaviour (Tinkle et al., 1970; Carothers, 1984; Anderson & Vitt, 1990). Sexual dimorphism in morphology, behaviour and colour pattern also indicate that sexual selection might be associated with competition for food between sexes (Schoener, 1967; 1977; Teixeira-Filho et al., 2003; Verrastro, 2004). Intersexual differences in body size can reveal impacts of ecological and evolutionary pressures on males and females differently in a population (Howes & Lougheed, 2007). Moreover, sexual dimorphism may be displayed in other traits such as the size of appendages (heads, tails and limbs) or scalation and colour (Gienger & Beck, 2007).

Morphologic sexual dimorphism in reptiles has been reported for lizards (Fitch, 1981). Sexual selection tends to ensure that males are larger than females (Vitt & Cooper, 1985), for example in males of territorial iguanids (Stamps, 1977; Fitch, 1980). However, in some forest iguanids such as *Enyaloides, Enyalius, Polychrus* and *Urostrophus*, the opposite can be observed with larger females than males (Fitch, 1981). Sexual dimorphism may also be associated with feeding ecology because differences in body and head size can reduce intersexual competition for food resources (Schoener, 1967; 1977). Thus, it is expected that sexual dimorphism can reflect differences in a species' diet.

The trophic relationships of lizards, their environments and foraging mode are important to understand a species' ecology. Diets may vary seasonally, ontogenetically and intersexually, and may differ depending on foraging strategies (Huev & Pianka, 1981; Dunham, 1983; Pianka, 1986; Vitt et al., 1996b). According to Optimal Foraging Theory - OFT (Schoener, 1971; Pianka, 1986), niche size increases as prey availability decreases in the environment, leading to a generalist diet; but, niche size decreases as resources increase, thus resulting in a specialist diet. Subsequently, the composition of an arthropod resource may lead to either generalist or specialist diets. In Atlantic forest biomes high availability of arthropods is common due to high diversity and abundance of species and a range of microhabitats. Thus, if arthropod diversity is high one could expect diet

specialisation. According to OFT, it could be expected that predators would have a specialist diet in such an environment. However, if there is low abundance of some arthropod species then it could be expected that lizards would be generalists.

In order to test this hypothesis, we analyzed the feeding habits and morphology of a Neotropical lizard population from the Atlantic forest, Brazil. Envalius Wied, 1821 lizards are diurnal. insectivorous, and occur in the Amazon region to the country's east coast, in Atlantic forest biome (Etheridge, 1969; Vanzolini, 1972; Jackson, 1978; Vitt et al., 1996a). Information about this genus is sparse (Van Sluys et al., 2004; Lima & Sousa, 2006; Barreto-Lima, 2009) and all species found from Brazil are endemic. Envaluus perditus Jackson (1978) from southeastern Brazil is insufficiently known (Lima & Sousa, 2006). The objective of this paper was to assess patterns of morphologic traits and diet of *E. perditus*. We analysed the kind of feeding habits developed, sexual dimorphism and if there was intersexual and temporal segregation in dietary composition of this species.

MATERIALS AND METHODS

The Santa Cândida Municipal Biological Reserve - SCMBR (21°45'S, 43°20'W), in Juiz de Fora, in the State of Minas Gerais, is a secondary urban forest fragment (113 ha) with typical Atlantic forest vegetation. Altitude ranges from 760 to 960 m and the forest is Montana Semidecidual Stational (after Lafetá, 1998). The climate is Cwa mesothermic-type (Köepen's Classification) showing an average annual rainfall of 1547 mm and an average annual temperature of 19.4°C. The seasons are hot and humid (HH), from the end of September (beginning of rainfall) through March of the following year (end of rainfall), and cool and dry (CD) from April through mid-September. We took 60 trips to SCMBR, from October 2003 to November 2004 to collect data. Data were gathered during mornings and afternoons. The sampling sites were chosen at random distances between 200 and 500 m of each other. Climate was recorded by the Universidade Federal de Juiz de Fora (UFJF) Climate Station, 3.11 miles from the study site.

We caught lizards by pitfall trapping and weighed them on a manual scale (0.10 g accuracy). The snout-vent length (SVL), mandibular length (JL), mandibular width (JW) and abdomen width (AW) were recorded with a manual caliper (0.1 mm accuracy). Tail length (TL) was measured with a ruler (to the nearest 1.0 mm).

We performed Mann Whitney U-tests to verify differences among the lizards' gender on morphometry. The above statistic was used as data were non-normally distributed. Sexual maturity was determined by SVL recorded and/or by gonadal ageing. We also described the lizards' general colour pattern before releasing them at the capture sites. Both adult and immature lizards were caught.

A collection of lizards was euthanized, fixed in 10% formalin, and stored in 70% ethanol. Specimens were dissected and their stomach contents removed before being deposited in the Herpetological Collection of UFJF. All other specimens were sampled using the "gastric-suction" method (Barreto-Lima, 2009). Before diet analyses we compared the methods used for collecting stomach contents (paired t-test), but no significant differences were found. Therefore it was possible to analyze both groups together (N: t = 1.86, P = 0.08; MA: t = 1.27, P = 0.22; VOL: t = 1.45, P = 0.16).

We analysed and classified all stomach contents (arthropods), under stereomicroscope to order or family (according to Buzzi, 2002). Stomach contents were then preserved in 70% ethanol. We did not consider dry decomposing plants as food and also removed sand and non-identifiable material.

From each food category we recorded the frequency (N = number of items), the frequency of occurrence (OF = how many times the same item showed in the stomachs), the mass in g (MA) and volume in mm³ (VOL). Depending on the statistical nature of the data we used Spearman Rank correlation or Pearson's t to analyze correlation among the lizards' SVL, mandibular length and width versus the highest VOL of prey eaten. We performed ANOVA tests for each diet variable (N, MA and VOL) versus genders or collection periods (2003 and 2004). All tests used Biostat 5.0 software

(2007) to a significance of $\alpha = 0.05$. We recorded MA with a digital analytical scale (0.0001 g accuracy) after withdrawing any excess liquid with tissue. For VOL, we used the perfect spheroid modified equation (Vitt et al., 1996b), where: VOL = $4/3\pi$ (length/2) x (width/2)².

The main diet items were those that showed the highest values in I(x) Importance Index (see Howard et al., 1999) for N, OF, MA and VOL variables. I(x) was calculated to assess the relative importance of a prey type in the whole diet. In the original equation, N, VOL, and OF are considered together to reduce index influence when fewer variables are used. The equation produced 0 to 1 values, representing the relative importance of a certain food item into the diet. In this study, we introduced MA variable in our analyses. Using all the variables together made the following equation possible:

$$I[x] = (n/N) + (v/V) + (f/F) + (m/MA) / 4$$

We carried out a survey on invertebrates in the area and calculated their Dominance Index (DI = number of individuals of a specific taxon/total number of individuals of a site x 100) and Occurrence (OI = number of samples where a taxon had been recorded/total number of samples recorded within a site x 100). In DI there are classes: from 0% to 25% = accidental; 25% to 50%= accessory; 50% to 100% = dominant. In OI, the classes are: from 0% to 25% = accidental; 25% to 50% = accessory, and from 50% to 100% = constant. This index combination allowed us to classify prev into: common, which is constant and dominant; intermediate, constant and accessory, constant and accidental, accessory and accidental, accessory and dominant; rare, accidental and accidental (Scatolini & Penteado-Dias, 2001). Jacobs Electivity Index I(EJ) was calculated with each type of prey found in the stomach and in the environment in order to verify the quantitative importance between them:

$$D = r - p / r + p - 2r$$

Here r and p are the percentage of diet and environment items respectively. Prey is avoided by a predator when D values are between -1 and approaching 0 (negative electivity) while it is selected when D values are between more than 0 to +1 (positive electivity). A value equal to 0 suggests null electivity.

RESULTS

Collecting

Twenty-eight invertebrate types were found in the environment (Table 1). Orthoptera, Coleoptera, Hymenoptera and Aranea were the most frequent but only Orthoptera was common. We collected 52 *E. perditus*, being successful during HH (Oct-Dec/2003, Sep-Nov/2004) only. At the beginning of HH seasons we found juvenile lizards as well as pregnant females during minimum rises in temperature.

Morphology

Few juveniles were collected (SVL = 34.4 ± 0.33 , range = 29.0-42.0 mm, n = 14). The adults' morphological data and mass/gender data (SVL = 74.7 ± 7.1 , range = 63.0-93.0 mm, n = 38) are shown in Table 2. Body differences were found (SVL: U = 29.0, P < 0.0001, JW: U = 84.0, P = 0.0238, TL: U = 80.5, P = 0.0177, AW: U = 48.5, P = 0.0007 and MA: U = 24.0, P < 0.0001) according to gender, except for JL (U = 109.5, P = 0.1442).

Colour

Males are leaf-green on their backs, displaying sky-blue colour down below their necks and irregular dark or light spots on their lower limbs. They might display an orange-yellow colour on their lower limbs (witnessed during reproductive time). Females are brown with dark symmetrical or asymmetrical spots along the paravertebral region, locomotion limbs and tail. They may display parallel white or off-white lines along their backs, from their heads to the tail base, and horizontal or irregular moss-green or brown traces up on their heads. On their backs, small off-white spots and/or short lines may come down from their necks. In both genders, the abdomen colour ranges from off-white to light brown and the tail exhibits scattered irregular dark spots (more evident in females).

Class	Order	Ν	DI (%)	OI (%)	Classification
Arachni	da				
	Araneae	111	5.18	100	Intermediate
	Opiliones	30	1.40	75.0	Intermediate
	Pseudoescorpiones	08	0.37	31.2	Intermediate
	Gamasida	03	0.14	6.2	Rare
	Ixodida	01	0.04	6.2	Rare
	non-identified	11	0.51	25	Rare
Diplopo	oda				
1 1	Polydesmida	03	0.14	6.2	Rare
	Spirobolida	17	0.79	37.5	Intermediate
	Spirostreptida	03	0.14	12.5	Rare
	non-identified	18	0.84	12.5	Rare
Ellipura	(Para-Insecta)				
P	Collembola	02	0.09	12.5	Rare
Insecta					
mseeta	Blattariae	21	0.98	56.2	Intermediate
	Coleoptera	432	20.20	87.5	Intermediate
	Dermaptera	11	0.51	12.5	Rare
	Diptera	01	0.04	6.2	Rare
	Isopoda	27	1.26	43.7	Intermediate
	Hemiptera	65	3.03	62.5	Intermediate
	Homoptera	01	0.04	6.2	Rare
	Hymenoptera				
	Formicidae	199	9.30	87.5	Intermediate
	Larvas	36	1.68	18.7	Rare
	Lepidoptera	06	0.28	18.7	Rare
	Neuroptera	01	0.04	6.2	Rare
	Odonata	01	0.04	6.2	Rare
	Orthoptera	1124	52.50	100	Ordinary
	Pupas	01	0.04	25	Rare
Total		2142	100	-	-

 Table 1. Invertebrates collected in Reserva Biológica Municipal Santa Cândida, Juiz de Fora, Minas Gerais, between

 2003 and 2004. Data on individuals number (N), Dominance Index (DI), Occurrence Index (OI) and classification of orders according to indices. DI = relative frequency (%).

Diet

We recorded 206 items in 14 prey categories. Five of 38 stomachs analyzed were empty. Two stomachs contained lizard scales. Formicidae and Isoptera were more consumed in N, insect larvae in OF, insect larvae and Orthoptera in MA, and Isoptera and Orthoptera in VOL. The main prey items I(x) were Formicidae, insect larvae, Isoptera and Orthoptera. Isoptera, Mantodae and insect eggs were the highest I(EJ). We did not find any significative correlation among the lizard SVL and the highest VOL of prey eaten ($r^{s} = 0.0959$, P = 0.5956, n = 33), neither among the jaw length and width of lizard versus the highest VOL of prey ($r^{s} = -0.0876$, P = 0.6278; P = 0.3355, P = 0.562, n = 33, respectively). According to gender, the highest I(x) were Formicidae, Isoptera, Orthoptera and insect larvae (Table 3).

Differences in diet according to gender were not found (N: F = 1.3782, P = 0.2930; MA: F = 0.0208, P = 1.0000; VOL: F = 0.0271, P = 1.0000, n = 12). In 2003 and 2004 the highest I(x) were Formicidae, Isoptera, insect larvae, and Orthoptera. There were differences in diet between the years to MA (F = 5.4158, P = 0.0037, n = 12) and VOL (F = 10.1764, P = 0.0003, n = 12), except for N (F = 1.1118, P = 0.4287, n = 12).

	Males (1	n = 26)	Females	(n = 12)
	Avg. SD	Range	Avg. SD	Range
SVL	71.5 ± 4.6	63.0 - 81.0	81.8 ± 6.5	73.0 - 93.0
JL	14.4 ± 1.9	12.0 - 18.0	15.3 ± 1.6	13.0 - 18.0
JW	12.1 ± 1.1	10.0 - 14.0	13.3 ± 1.4	12.0 - 16.0
TL	156.8 ± 14.9	136.0 -172.0	170.9 ± 13.9	152.0 - 200.0
AW	11.3 ± 1.8	0.9 - 15.0	15.3 ± 3.3	10.0 - 20.0
MA	8.3 ± 2.0	4.0 - 12.5	15.4 ± 4.3	8.0 - 22.0

 Table 2. Morphological data on 38 adult *Enyalius perditus* collected in Reserva Biológica Municipal Santa Cândida,

 Juiz de Fora, Minas Gerais, in 2003/2004. Snout-vent length (SVL), jaw length (JL), jaw width (JW), tail length (TL),

 abdomen width (AW) in mm and mass in g (MA).

DISCUSSION

Climate Rain scarcity may have influenced availability and abundance of prey because we collected fewer and less diverse arthropods under dry conditions. It is known there is a relation between rainfall, primary productivity and abundance of insects in tropical habitats (Pianka, 1986; Vitt, 1990). Juvenile lizards, gravid females and adult males observed during HH indicated that the population was most reproductively active during the hottest seasons. There was strong evironmental influence on the lizards' reproductive tactics (Colli, 1991), perhaps in response to greater productivity and reproduction by arthropods in the area. The absence of lizards from February-August 2004 may have been caused by heavy rainfall (Feb) and a fall in temperature in CD. This could have restrained physical activity of

the lizards. During CD *E. perditus* may decrease its activity or enter a seasonal torpor as suggested for other *Enyalius* spp. in Brazilian winter (Grantsau, 1966).

Morphology

As expected, females were heavier (MA) and larger in body size (SVL), tail (TL), and other measurements (JW and AW). As observed in this study, and for other continental forest genera, male *Enyalius* spp. lizards are smaller than females (Fitch, 1981; Jackson, 1978; Vitt et al., 1996a; Teixeira et al., 2005). For *E. perditus*, Sturaro & Silva (2010) revealed no sexual dimorphism in the majority of morphometric characters, except in SVL. Typically, sexual dimorphism is affected by sexual selection (Carothers, 1984; Vitt et al., 1996b) or female reproductive strategy in lizards

Dietary Category			Males (n	= 22)				Females	(n = 11)	
	N(%)	OF(%)	MA(%)	VOL(%)I (x)	N(%)	OF(%)	MA(%)	VOL(%)I (x)
Araneae	5.6	22.7	3.5	7.4	0.0978	3.7	27.3	6.6	7.1	0.0737
Blattariae	0.8	2.2	2.6	3.4	0.0278	2.5	18.2	14.0	23.9	0.1207
Coleoptera	2.4	6.6	4.2	4.6	0.0614	2.5	9.1	1.2	2.1	0.0245
Dermaptera	0.8	2.2	3.6	1.4	0.0256	1.25	9.1	3.8	3.0	0.0301
Diplopoda	3.2	8.8	5.5	2.6	0.0735	1.25	9.1	0.6	0.5	0.0150
Diptera	0.8	2.2	0.01	0.01	0.0130	0	0	0	0	0
Formicidae	45.2	54.5	18.5	14.6	0.3319	37.5	27.3	5.5	3.8	0.1470
Isopoda	2.4	6.6	1.6	1.2	0.0469	1.25	9.1	.2	1.1	0.0186
Isoptera	28.6	9.1	12.3	23.1	0.1822	17.5	45.5	7.8	20.9	0.1657
Insect larvae	4.0	22.7	14.6	15.7	0.1423	5.0	36.4	45.0	17.1	0.2078
Mantodea	0.8	2.2	5.6	3.0	0.0347	0	0	0	0	0
Orthoptera	5.6	22.7	27.9	22.6	0.1511	5.0	27.3	11.9	19.8	0.1218
Insect eggs	0	0	0	0	0	22.5	9.1	2.5	0.6	0.0739
Total	~100	-	~100	~100	-	~100	-	~100	~100	-

Table 3. Percentage values of *Enyalius perditus* diet (n = 33) collected in RBMSC, Juiz de Fora, Minas Gerais, in 2003 and 2004, presenting frequency (N), occurrence frequency (OF), mass in g (MA), volume in mm³ (VOL) and Importance Index I(x) for each type of prey consumed in accordance with the lizards' sex.

(Tinkle et al., 1970). Larger females have increased fecundity because they can accommodate more eggs inside their bodies (Trivers, 1972; Arak, 1988). However, differential mortality between gender is more often due to increased activity and predation risk exhibited by males when searching for mates (Anderson & Vitt, 1990). This factor could influence sexual dimorphism observed in E. perditus. The size of prey eaten by lizards may also be associated with gape size. However, we did not find any positive correlation between size of lizard and prey (in VOL) as is exhibited for E. leechii (Vitt el al., 1996a), E. bilineatus (Zamprogno et al., 2001) and E. brasiliensis (Van Sluys et al., 2004). E. perditus seems to not select prey based on size because there is no relationship between SVL, head length and prey length (Sturaro & Silva, 2010). To the contrary, Sousa & Cruz (2008) found opposing results for *E. perditus*, but this may have been because they used the prey's average length and not VOL in analyses. In this study, Isoptera was the most consumed prey (in VOL). Termites are small invertebrates with little variation in size. and as such they are easily consumable by both small and mid-sized lizards (Teixeira-Filho et al., 2003). Interestingly, we found Orthopteran nymphs in the stomachs of two juvenile lizards, identical in size and volume to those found in adult lizards

Colour

Sexual selection in *E. perditus* is possibly related to other factors such as coloration (Sturaro & Silva, 2010). Male lizards shift colour to a darker shade of brown compared to females when copulating (Lima & Sousa, 2006), stressed or sick in captivity, or when exposed to mild temperatures. *E. perditus* colour dimorphism (Jackson, 1978) may be the result of selective pressures for the species', intra and intersexual recognition and/or associated with the length of time evolved in a particular microhabitat (Rocha, 1994; Vitt et al., 1996a). Colour dimorphism has frequently been identified among *Enyalius* spp. (Jackson, 1978; Vitt et al., 1996a; Rodrigues et al., 2006; Zatz, 2002; Sturaro & Silva, 2010).

Diet

Enyalius perditus diet diversified with higher

intake of highly active prey (Formicidae and Orthoptera), low mobility prey (larvae), and randomly distributed prey (Isoptera). This possibly suggests opportunistic foraging. Ants were also consumed, suggesting a prey item costing low energy expediture (Barreto-Lima et al., unpublished data). Isoptera, Mantodae, and insect eggs were important for I(EJ) because they were collected in the stomach content rather than in the environment. Nonetheless. Mantodae and insect's eggs were found in only one stomach, so were not significant to the total diet. Orthoptera and Formicidae were not important for I(EJ), probably because they were available in higher abundance. A priori, Enyalius was considered a generalist predator of soil arthropods (Zamprogno et al., 2001; Van Sluys et al., 2004; Teixeira et al., 2005). However E. perditus was considered an active forager with a broad diet but it still exhibits some prey preferences (Sousa & Cruz, 2008; Sturaro & Silva, 2010).

In this study, E. perditus feeding habits do not appear to be typical of a forest specialist and lean more toward opportunistic predation. Lizards in such an intermediary category, adopting both strategies (active and ambush forager) are defined as cruising foragers (Pough et al., 1999). As expected by OFT (Schoener, 1971; Pianka, 1986) niche size increases when prey availability decreases in an environment and this thus leads to a generalist diet (herein only Orthoptera was a common active prey). One possible explanation for these findings is anthropic alteration to the habitats (i.e., the study area, SCMBR was a formerly disturbed coffee plantation). In native forest areas, Sousa & Cruz (2008) reported that E. perditus consumed more Isopoda, Formicidae and insect larvae, than reported herein. However, in other studies larvae of Lepidoptera, Araneae, Formicidae and Isoptera were the most abundant food items in E. perditus' diet (Sturaro & Silva, 2010).

The SCMBR urban forest physiognomy differs from typical natural forests in the region because anthropic actions have altered the local arthropod fauna's natural balance. Thus, as populations of *E. perditus* are in distinct areas, prey availability influences dietary composition (C.F.D. Rocha, pers. comm.). In *E. leechii* diet, Isoptera and insect larvae were dominant (Vitt et al., 1996a), while in E. catenatus diet, Orthoptera were dominant (Grantsau, 1966). In E. bilineatus Orthoptera, Homoptera, Hymenoptera, Blattariae, Hymenoptera and Dyctioptera were recorded (Vanzolini, 1972; Zamprogno et al., 2001), while E. brasiliensis ate Formicidae, insect larvae, Orthoptera and Isoptera (Van Sluys et al., 2004). It is likely that Envaluas consume their shed skin to reingest proteins (Vanzolini. 1972: Vitt et al.. 1996a: Teixeira et al., 2005).

Herein, E. perditus could be considered semiarboreal because we observed the lizards on the ground (pitfalls) and by scanning tree branches. Overlapping of resources in the population's diet is likely to exist because there is neither intersexual difference in the diets (in N, MA and VOL), nor a positive correlation between the preys highest VOL and the lizards SVL. This lack of a positive correlation eliminates possible differences in the population's diet that could result from resource sharing due to size (Heideman & Bates, 1999). Sturaro & Silva (2010) also observed numeric and volumetric diet overlap between males and females. However, Sousa & Cruz (2008) saw differences in E. perditus' diet according to gender. In this case, gender differences in diet may be explained as a local adaptation to prey availability rather than a characteristic of the population.

Although there is evidence for sexual dimorphism in *E. perditus*, in this study we observed intersexual trophic similarities in diet. This is to the contrary with previously observed tendencies to resource partition by genders in many lizard species (see Schoener, 1967; 1977; Carothers, 1984). The consumption of similar food items across samples in our area of study does not seem to support competition as a major influence on sexual dimorphism. At SCMBR, most arthropods were terrestrial and we also noticed *E. perditus* foraging among litter in the reserve.

We also found decomposed vegetation inside stomachs. Apparently, this suggests accidental intake during predation as previously noted for *E. perditus* (Sturaro & Silva, 2010), *E. brasiliensis* (Van Sluys et al., 2004) and *E. bilineatus* (Zamprogno et al., 2001). Our dietary records showed alterations between years (MA and VOL). Diversity of prey in the diet may vary between species, time, area and seasonal availability of prev type (Pianka, 1986) or in adaptation to an environment (Colli, 1991). It is also possible that the number of lizards examined between years (13 in 2003 and 20 in 2004) influenced these observed differences in prev MA and VOL. Furthermore. larvae abundance should correspond to insect productivity in the area and coincide with a successful collection period (HU). For Isoptera, the collections during HU coincided with termite nests release of flying adults followed by their agglomeration on the ground for nesting (F. Prezoto, pers. comm.). However, Formicidae and Orthoptera were abundant at the site throughout the period of study.

As expected, in the anthropized forest area with lower prey abundance *E. perditus* was considered an opportunist predator of terrestrial arthropods, having Formicidae, Isoptera, insect larvae and Orthoptera as dominant prey items. However, sexual dimorphism shown by *E. perditus* in this study disagreed with previously recorded accounts because there were intersexual trophic similarities. This suggests there is possibly no differences in resource partitioning of food by gender.

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Amazonian frog diversity and microhabitat use

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ABSTRACT - Upper Amazonian forests offer some of the highest species diversity in the world due in part to their complex habitats created by fluctuating water levels. In the Pacaya-Samiria National Reserve within the upper Amazonian forest of Peru, forty species of anuran belonging to seven families were recorded in 2009 and 2010 over forty survey days. A species accumulation curve indicated that most species present were detected after ten days of surveying. On land, frogs were most frequently observed among leaf litter. In the river, floating rafts of vegetation may be an important mechanism for the dispersal of frogs.

THE Amazon rainforest contains some of the greatest species diversity on Earth (Salo et al., 1986; Osborne, 2000; Bodmer, 2008). It is a complex ecosystem combining different strata from emergent layer through to canopy, shrubs and forest floor. This wealth of niches has enabled many species to evolve specialist adaptations to their environment. Consequently a huge diversity of amphibian and reptile species exist in the Amazon, with over 250 amphibian and reptile species described as "commonly seen" (Bartlett & Bartlett, 2003).

Surveys indicate that the upper Amazonian forests offer high species diversity due to complex habitats created by fluctuating water levels (Salo et al., 1986; Gentry, 1988; Bodmer, 2008). Gentry (1988) surveyed a series of 1 ha plots in Peru, and found 580 individual trees representing 283 species per plot. The Amazon rainforest would not function without the Amazon river which forms at the confluence of the Maranon and Ucavali rivers. These rivers border the Pacaya-Samiria National Reserve, a 8,042 km² protected area located in the upper Amazonian forests of Loreto, Peru. This region contains one of the highest anuran diversities in the world. Rodriguez & Duellman (1994) describe 112 species from the Iquitos region alone. The number of anuran species in this area is constantly increasing as new species are discovered (Perez-Pena et al., 2010). The Pacava-Samiria reserve has been degraded in the past through overhunting, deforestation and overfishing (Bodmer, 2008). However, wildlife monitoring in the reserve has noted increases in woolly monkeys *Lagothrix lagothrica*, black caiman *Melanosucus niger*, manatees *Trichechus inunguis*, dolphins *Inea geoffrensis* and macaws (Bodmer, 2008).

Despite ongoing monitoring of wildlife in this reserve, little research on diversity and populations of amphibians has been published. The aims of this research were to create a baseline anuran species list for the Pacaya-Samiria reserve and describe the habitat and microhabitat use by them.

MATERIALS AND METHODS

Site Description

This study was undertaken in the Pacaya-Samiria National Reserve, a site with a complex ecosystem. The reserve does not have strictly defined wet and dry seasons and more often has high and low water seasons. As a result of extreme seasonal water changes 92% of the reserve comprises low lying flooded forest know as varsea (Myers, 1990; Talling & Lemoalle, 1998). Inundation and run-off of tannins from trees likely creates the blackwaters of the Samiria River (Bodmer et al., 2010). Periodically, the forest becomes flooded with white water from the Maranon river. The sediment from this water is dropped and tannins from decomposing leaves are taken in. This water then flows back out of the forest into the Samiria River as tannin rich blackwater (Bodmer, pers. comm.).

The Samiria River is an old channel of the Manranon River, therefore the Samiria river bed contains nutrient rich alluvial soils (Kvist & Nebel, 2001). This hydrological system, combined with the alluvial soils, helps create an environment that is very nutrient rich and therefore able to support a diverse range of species across many taxa.

Methods (2009)

Surveys were carried out adjacent to a location known as PV3, a guard post on the Samiria River, at Hungurahui. Land (walking) and river (canoe) transects were conducted within the vicinity of PV3. Data were collected over 18 days between the 30 May to 16 June 2009. During this time 104 transects of 100 m were completed in 52.5 hours. Transects were alternated between land (52 surveys) and river (52 surveys) with equal numbers at day and night in a variety of habitats and temporal zones. River transects were alternated between banks, with a GPS used to calculate distance travelled. For land transects, a tape measure was used with random numbers applied to a compass to determine the direction of travel. Sampling was undertaken no higher than 2 m from the ground or river surface and transect width was 4 m. Day surveys began at 08:00 lasting until approximately 13:00. Night surveys were from 19:00 to 22:00. A team of three to four people walked each land transect and canoed each river transect using a visual encounter survey method (VES) which has been shown to give a good representation of species in tropical forests over a short time period (Doan, 2003). There was no time limit on each transect. They were travelled at the same speed of 0.5 km an hour. Each individual amphibian was captured to collect data. Date, time and transect number were recorded as well as habitat, microhabitat, and substrate. The individuals were then measured (1 mm precision) and weighed (0.1 g precision). Additional factors including temperature, rainfall, detection method, light level and ecologically relevant notes (e.g. sitting on a foam nest) were also recorded. Identification was undertaken using three guide books; Rodriguez & Duellman (1994), Bartlett & Bartlett (2003) and Duellman (2005). Where possible identification was confirmed by local experts.

Methods (2010)

Data were collected from the 15 June to 10 July 2010 (22 survey days). A total of 31 sampling transects was undertaken comprising four permanent land and five permanent river transects, each of 1000 m, surveyed both nocturnally and diurnally. A total of 64 hours of survey was completed. Transects began at 10:00 for the dawn transects and 20:00 for the night transects. VES method was used. The land surveys involved scanning leaf litter and vegetation whilst walking along the transect, using sticks to tap the leaf litter during the day and using torches to spot frogs at night. River surveys involved using torches to scan the riverbank and floating vegetation. All other methods were the same as described for 2009.

RESULTS AND DISCUSSION

Diversity of Amphibians

Forty amphibian species belonging to seven families were recorded in Pacaya-Samiria during 2009 and 2010. They included; Arobatidate (1 species), Bufonidae (3 species), Dendrobatidae (2 species), Hylidae (23 species), Leptodactylidae (8 species), Microhylidae (1 species), Strabomantidae (2 species). Appendix 1 shows a full list of species and the corresponding years in which they were recorded. The highest number of species was recorded in 2009 (29 species). Twenty-seven species were recorded at the same site in 2010. Between these two studies a total of 845 anurans were caught in just 40 days of surveys.

The species list compiled from the 2009 and 2010 research shows possible absences as well as new discoveries in some species. However, the differences in methods and timing make comparisons in abundance difficult without longterm monitoring. Nevertheless, the Pacaya-Samiria reserve has an extremely high anuran diversity (40 species recorded), which can be compared with other anuran hotspots. For example, 52 amphibian species have been recorded in just 45 hectares of Costa Rica (Kubicki, 2010), 27 species representing 5 families were found in Borneo (Keller at al., 2009) and studies on woodlands in western Tanzania found 4247 individuals representing 28 amphibian species (Gardner et al., 2007). The Gibraltar Range National Park in Australia is also

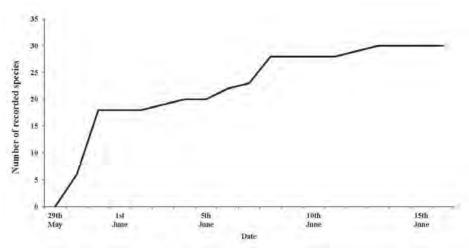


Figure 1. The species accumulation curve for the 2009 Pacaya-Samiria study

home to 30 anuran species (Mahony, 2006). These studies all had longer survey periods than that of the Pacaya-Samiria research and are therefore more extensive. Despite these caveats, 40 amphibian species were recorded in just 40 days; representing a higher diversity than three of these four studies. Fig. 1 shows the species accumulation curve for the 18 days spent in the field in 2009. The curve stabilised after 10 days of surveying. This suggests the majority of species present in the habitats surveyed had been observed.

Microhabitat Use

Fig. 2 shows the number of individuals of the five most abundant species found in the terrestrial habitat in each of the three main micro-habitats on the forest floor (2009 data only). The five species were found in differing frequencies across the three microhabitats suggesting differential usage (Chi-squared = 24.09, df = 8, P < 0.01). Most frogs were found in leaf litter. *Leptodactylus discodactylus* showed no preference for a single habitat type. *Rhinella margaritifera* was most commonly found in the leaf litter.

The high diversity of species may present the possibility of resource partitioning on a spatial scale. Many microhabitats were available within the terrestrial habitat including leaf litter, bare ground, puddles, tree trunks and fallen logs. When foraging, frogs may utilise a range of microhabitats as they travel through their range. Leaf litter was the microhabitat utilised most often in this study, a finding supported by Morales & McDiarmid (1996). Leaf litter may reduce the risk of detection by predators (Vonesh, 2001). *Rhinella margaritifer* and *Rhinella daphillis* were often recorded in the leaf litter and have coloration and morphology that resembles leaves of the region (Marent, 2008).

All but one dendrobatid species found in 2009 were active in open spaces during the day. This is commonly recorded behaviour for frogs of the family as they produce toxins which are unpalatable to potential predators; a point broadcast by their striking colours (Symela et al., 2001). A single *Ameerega trivittata* was observed on the same log for three consecutive days. As dendrobatids defend small territories that contain good breeding sites (Poelman & Dicke 2008), this *Ameerega trivittata* may have been the same individual, however, without marking for recapture this could not be confirmed.

The Floating Meadows

Due to the high level of flood water in Pacaya-Samiria in 2009 the only habitat available on the river was floating meadow (2009 data only). Fig. 3 shows the number of individuals representing each species in each of the three main microhabitats found on the floating meadow (Fig. 4 illustrates these microhabitats). The floating meadow habitat and its microhabitats were able to support a large number of species. Sixteen species were found

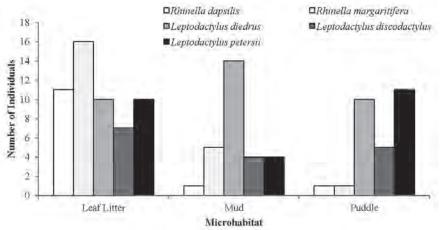


Figure 2. The number of individuals, of each species, recorded on the three main microhabitats on the land transects (2009).

using this habitat, while both Hödl (1977) and Goulding (1989) recorded 15 species on floating meadow at different Amazon sites. These meadows are created from extensive macrophyte stands that grow along the banks of rivers and in lakes (Schiesari et al., 2003). In some parts the meadows covered the entire water channel from one bank to the other, a feature that could aid dispersal across the river. The floating meadows may have been formed at a lake up-river from the study site and therefore facilitate dispersal downstream as well. However, further research is required to confirm this.

The water lettuce microhabitat was dominated by Sphaenorpyhchus dorisae and Sphaenorhynchus

lacteus. Both of these were found most often on this microhabitat, with small numbers recorded in the other two microhabitats. *S. lacteus* was found mainly on this microhabitat possibly due to its morphology. *S. lacteus* was one of the largest species found on the floating meadows. It also lacks adhesive disks on its fingertips (Rodriguez & Duellman, 1994). The emergent vegetation and water hyacinth were very spindly and weak and therefore may only be able to support smaller hylid species.

Calling site partitioning has been observed on floating meadows (Hödl, 1977). Four of the species recorded herein also featured in Hödl's (1977) study, with each observed frog calling from one

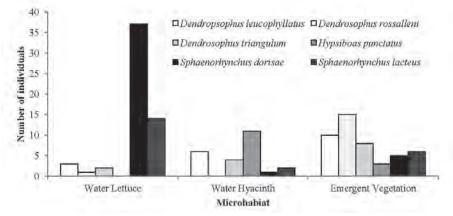


Figure 3. The number of individuals, of each species, recorded on the three main microhabitats on the river transects (2009).



Figure 4. A small section of the floating meadow habitat connected to the flooded forest. In this photograph water lettuce, water hyacinth and emergent vegetation are all present.

particular microhabitat, possibly attracting mates for breeding. In addition to calling adults, froglets were also observed on the floating meadow habitat. Many species in the central Amazon tropical forest breed all year round (Hödl, 1990). Frogs may also have been exploiting the abundance of insect prey available on the floating meadow habitat (Schiesari et al., 2003). The meadows grow very rapidly thus producing a lot of detritus and shelter in the root zone that provides suitable habitat and food for a wide variety of invertebrates (Schiesari et al., 2003). Many frogs observed on the floating meadow habitat were hylid species that would usually be expected to be found in the canopy. Thus this habitat could offer a rare opportunity to study their ecology.

Floating meadows are not permanent habitats. Sections break away, creating floating rafts of vegetation carried down river after rainfall (Schiesari et al., 2003) (Fig. 5). This transport of individuals can be very important to downriver dispersal, facilitating gene flow (Schiesari et al., 2003). Species found on these floating rafts include Rhinella marinus, Leptodactylus leptodactyloides, Dendropsophus leuchophyllatus, Hypsiboas punctatus and Sphaenorhynchus carneus (Schiesari et al., 2003), all of which were present in this habitat during this study. A further four species were found on floating rafts by Schiesari et al. (2003). However, their survey methods were more intensive. Surveying included eight floating rafts collected in their entirety, with all vertebrates counted and identified. These rafts were collected in Brazil on the Solimões River, which prompts the question of whether such rafts could travel this far. Schiesari et al. (2003) calculated that a vegetation raft could travel 4000 km in as little as 31 days. These rafts also have a great abundance of prey species as the submerged root zone of 1 m² of floating meadow will usually support over 500,000



Figure 5. Rafts observed floating down river transporting anuran species. This raft contained *Dendropsophus triangulum* and *Hypsiboas punctatus* individuals.

invertebrate individuals (Goulding, 1989). Therefore, rivers may not be barriers to the dispersal of terrestrial amphibians, but actually aid population dispersal.

Further impacts like disease should be considered potential threats to herpetofauna of floodplains, especially chytridiomycosis. If present in aquatic environments, infected frogs could spread the disease easily when they are breeding further downstream. The potential impact of climate change in the area could also threaten dramatic changes in the water levels and flooding patterns that may have far-reaching impacts on amphibian diversity and abundance.

Further research would be required to fully investigate amphibian population trends in Pacaya-Samiria National Reserve. Such work will hopefully form the basis of a Ph.D. conducted by the senior author commencing September 2011, that seeks to assess the suitability of amphibians in tropical environments as indicator species.

ACKNOWLEDGEMENTS

I would firstly like to thank Dr. Richard Bodmer without whom this research would not be possible. I would also like to thank Professor Richard Griffiths for his support and guidance. A special thanks goes out to my field guide Renee, Ellie Passingham for reading a draft manuscript, all the DICE students who made the expeditions unforgettable and all who helped out in the field. Thanks to the Pacaya-Samiria Reserve for authorisation and permission to conduct this research, and to the following for helping to fund this project and for logistical support: Durrell Institute of Conservation and Ecology, Wildlife Conservation Society, Earthwatch Institute and Operation Wallacea. Finally a huge thank you to Phillip Camp for support throughout.

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APPENDIX

Numbers of individuals of each species observed in Pacaya-Samiria National Reserve.

Family	Scientific Name	2009	2010
Arobatidate	Allobates femoralis	1	2
Bufonidae	Rhinella dapsilis Rhinella margaritifera Rhinella marina	14 25 1	- 2 17
Dendrobatidae	Ameerega hahneli Ameerega trivittata	1 3	- 1
Hylidae	Dendropsophus haraldschultzi Dendropsophus leucophyllatus Dendropsophus parviceps Dendropsophus rossalleni Dendropsophus triangulum Dendropsophus allenorum Hypsiboas boans Hypsiboas fasciatus Hypsiboas geographicus Hypsiboas lanciformis Hypsiboas punctatus Osteocephalus buckleyi Osteocephalus cabrerai Osteocephalus leprieurii	- 20 - 16 15 - - 2 - - 14 1 1	2 - 5 7 58 1 9 2 1 7 22 - -

	Osteocephalus planiceps	-	1
	Osteocephalus taurinus	3	11
	Scarthyla goinorum	-	7
	Scinax ruber	1	-
	Scinax pedromedinae	-	19
	Sphaenorhynchus carneus	5	-
	Sphaenorhynchus dorisae	43	8
	Sphaenorhynchus lacteus	22	-
Leptodactylidae	Trachycephalus resinifictrix	-	2
	Leptodactylus andreae	3	9
Leptonetyman	Leptodactylus diedrus Leptodactylus discodactylus	43 16	- 24
	Leptodactylus hylaedactyla	6	6
	Leptodactylus leptodactyloides	13	265
	Leptodactylus mystaceus	5	-
	Leptodactylus pentadactylus	1	6
	Leptodactylus petersii	31	36
Microhylidae	Hamptophryne boliviana	2	-
Strabomantidae	Pristimantis altamazonicus Pristimantis carvalhoi	- 2	4 -

Evaluation of methods to separate brown and water frogs

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ABSTRACT - Methods given in the herpetological literature for distinguishing between the northwest European brown and water frog groups (*Rana* and *Pelophylax* respectively) are reviewed and evaluated. Published guidance contains inaccuracies that could create misidentifications. The unreliability of the method most commonly described for separating the two groupings, the presence/ absence of an eye stripe, is highlighted. The relative distance between the eyes and the degree to which eyes are upturned both reliably distinguish between the two groups. Differences in the shapes of dorsolateral folds also separate the two groups, but less unequivocally.

A N experienced herpetologist can distinguish between brown or water frogs on 'General Impression of Shape and Size' (GISS, occasionally written as 'gizz' or 'jizz'). It has, however, been the author's experience that a great deal of published guidance (including the most widely read) on the separation of these groups is inadequate or erroneous. This present work tests the accuracy of some of the published methods of separation and revises existing guidance.

The work focuses on northwest European species, comprising three indigenous brown frogs, the common frog *Rana temporaria*, the moor frog *Rana arvalis* and the agile frog *Rana dalmatina*, and three indigenous water frog types, comprising two species, the pool frog *Pelophylax lessonae* (formerly *Rana lessonae*) and the marsh frog *Pelophylax ridibundus* (formerly *Rana ridibunda*), and their hybrid, the edible frog *Pelophylax* kl. *esculentus* (formerly *Rana* kl. *esculenta*).

Colour is not always a reliable guide for separating the brown and water frogs. Water frogs often have areas of vivid green dorsally (hence the alternative name of green frogs). Although this colour intensity is not achieved in the brown frogs, some common frogs have a pale olive colouring dorsally. To compound the problem some pool and edible frogs can also be brown dorsally. In fact, the northern clade pool frog (the form native to Britain and Scandinavia) is always brown. The presence of a dorsal stripe for group or species separation is also unreliable and intraspecific variation occurs geographically. Juvenile and female pool and edible frogs often have a dorsal stripe as do many males. The occasional common frog and a great many moor frogs also have dorsal stripes (although these are often wider and less defined at the edges than those in the water frogs). In the marsh frog the striped condition seems to vary from population to population. In the southern Kent marshes and parts of the north Kent marshes, the author has not yet seen a striped marsh frog, while on Chetney Marshes in north Kent, striped individuals are common.

Behaviour can be a useful guide. Water frogs are usually found in, or close to, water (usually within 2 m). If they are on the banks when approached, they launch themselves into the water with surprisingly little splash. Brown frogs, such as the common frog, are mainly to be found in water only in very early spring (usually before the water frogs have even left hibernation) and are noisier and less streamlined in their entry into the water. They are also less nervous and can be more closely approached without causing them to panic.

The presence of paired vocal sacs (one either side of the head) in water frogs is a reliable guide but limited to males and, outside of the spring to early summer calling season, needs examination in the hand. Identification handbooks suggest various other ways of separating the two groups, which are summarised below.

Relative Distance Between the Eyes

Arnold & Ovenden (2002) describe the eyes of water frogs as 'close together' whereas those of brown frogs are 'well separated' (Fig. 1).

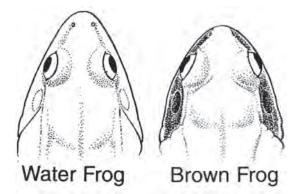


Figure 1. Illustration from Arnold & Ovenden (2002) depicting the difference in the relative separation of the eyes.

Inclination of the Eyes

Nöllert & Nöllert (1992) suggest that the eyes of water frogs appear to be more upward looking than those of the brown frog group.

Configuration of the Dorsolateral Folds

Fog et al. (1997 [in Danish]) suggest that there is a difference between the groups in the linear patterns of the dorsolateral folds (Fig. 2).

Presence/Absence of a Temporal Mask

The most enduring and widespread advice in the literature concerns the presence (in brown frogs) or absence (in water frogs) of a dark 'facial mask'. This is also variously described as a temporal mask or facial stripe. This advice has been given for over a century as a reliable means of separating brown and water frogs and can be found in even the most popular and frequently cited European amphibian and reptile identification handbooks (Mivart, 1874; Chihar & Cepika, 1979; Laňka & Vít, 1989; Arnold, Burton & Ovenden, 1978; Beebee & Griffiths, 2000; Arnold & Ovenden, 2002; Wycherley, 2003; Inns, 2009). Nöllert & Nöllert (1992) suggested less certainty, stating that brown frogs mainly, and water frogs rarely, possess a mask. Other guides (Matz & Weber, 1983; Ballasina, 1984) do not mention this method. Morrison (1994) stated that a temporal mask was a characteristic of brown frogs, however, this text was embedded among illustrations of seven common frogs, three of which had no temporal mask. The ideal case inferred

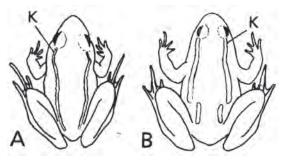


Figure 2. An Illustration from Fog et al. (1997) indicating differences in the form of the dorsolateral folds (K). A = typical brown frog, B = typical water frog.

by the literature is shown in Fig. 1, which shows the head of a typical water frog with no temporal stripe and a brown frog with a bold stripe. The dark stripe begins at the tip of the snout, runs through the nostril and stops at the anterior part of the eye. It then continues from the posterior part of the eye and passes diagonally downwards across the eardrum and towards the shoulder.

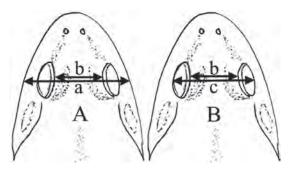
MATERIALS AND METHODS

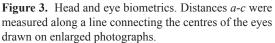
Relative Distance Between the Eyes

Photographs of 20 brown frogs and 20 water frogs were taken with a digital camera held directly over the subject's head. Measurements were made from enlarged photographs using a Vernier calliper. These included the width of the head a and the distance between the inner margins of the eyes b, both measured along a line taken through the centre of the eyes (Fig. 3). Distance a was divided by b to give the relative separation of the eyes in the two frog groups.

Inclination of the Eyes

To measure relative eye width visible from above (i.e. to test if the eyes of water frogs are more upward looking than those of brown frogs), the distance between the outer edges of the eyes c was measured (Fig. 3). The difference between c and b was divided by a ([c-b]/a). To test if the species were comparable in respect of head width (measured just behind the eye bulge), body length





and eye diameter, measurements were taken for 54 individuals and subjected to t-tests applied between the six species. As an additional test, measurements were taken from photographs of frogs taken head-on, close to water level. A horizontal line was created (longer black line, Fig. 4) (using Microsoft PhotoDraw v.2) and the photographs rotated until the lower margins of the eyes were aligned with this. A straight line was then drawn through the upper and lower eyelids at their widest point in each eye. A line perpendicular to this was drawn, through the horizontal. The inclination of both eyes from this horizontal was measured using a protractor, and the angles of inclination for both eyes were averaged. The results from the two groups were compared using t-tests.

Configuration of the Dorsolateral Folds

The form and linearity of the dorsolateral folds in photographs of sixty-six water frogs and fifty-one brown frogs were compared with the examples given by Fog et al. (1997) (Fig. 2). These photographs were mostly from the author's collection supplemented with a small number from the internet.

Presence/Absence of a Temporal Mask

The presence/absence of a dark facial mask was examined either with specimens examined in the field or, for the most part, using photographs, with no conscious bias in selection. Altogether 398 water frogs, consisting of five species were examined. Approximately half of the images were from the author's collection and the rest were from the Internet.

RESULTS

Relative Distance Between the Eyes

The mean ratio of head width *a* to the distance between the eyes *b* for water frogs was 1.65 (s.d. = 0.11) and for brown frogs 1.1 (s.d. = 0.08). The assertion in Arnold et al. (1978), Arnold & Ovenden (2002) that the eyes of water frogs are 'close together' compared to those of brown frogs was strongly supported (t = 16.9, p < 0.0005).

Inclination of the Eyes

There was a significant difference in the mean

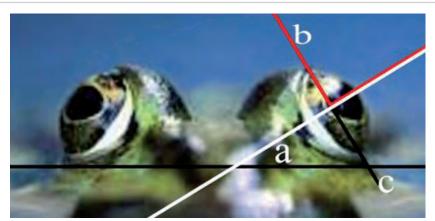


Figure 4. Measurement of angle of inclination of the eye. Using head-on photographs of frogs taken close to the water. Line b-c passes from the edge of the upper to lower eyelids at their widest point. The white line was drawn perpendicular to b-c and a was measured as the angle of inclination.

ratio (c-b)/a between water frogs and brown frogs (means = 0.27, s.d. = 0.04 and 0.13, s.d. = 0.04respectively, t = 11.44, p < 0.0005). This equates, on average, to approximately double the proportion of the eye width visible from above in water frogs, or about one quarter of the width of the head is taken up by the eyes in water frogs and just one eight of the width in brown frogs. There were no significant differences in head width relative to body length (snout to vent) proportions between the two groups (means = 0.32, s.d. = 0.036 and 0.326, s.d. = 0.03 for water and brown frogs respectively, t = -0.55, p > 0.50). There were also no significant differences in eye diameter relative to body length (mean = 10.01, s.d. = 0.6 and 9.96, s.d. = 0.5, t = 0.58, df = 54, p > 0.55). Hence differences in the width of eye seen from above were not due to differences in the size of eyes between the two groups, but due to the angle of inclination of the eves.

The results from the inclination measurements taken from head-on photographs also differed between the two groups (t = -10.27, P < 0.0005). The average inclination from the horizontal for brown frogs was 9° (s.d. = 5.1), while for water frogs the angle was 28° (s.d. = 3.0). The suggestion that the eyes of water frogs are more upward looking compared to those of brown frogs was, therefore, strongly supported. Neither the eye separation nor inclination results showed any overlap between the groups.

Configuration of the Dorsolateral Folds

The differences suggested in the dorsolateral fold patterning between the brown and water frog groups were also supported, although rather than the two forms given by Fog et al. (1997) a range of dorsolateral fold patterns was discernable. Nine variations are given in Fig. 5. There was strong agreement with the suggestion in Fog et al. (1997) that brown frogs display pattern A. Thirty brown frogs out of a total of 51 (59%) had this pattern which was not seen in water frogs. Fog et

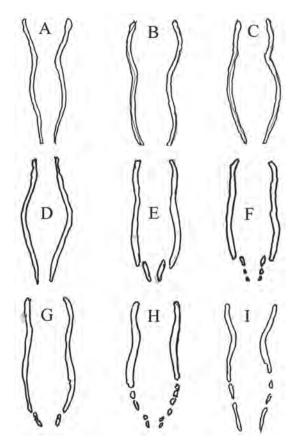


Figure 5. Dorsolateral fold pattern types across three water frog species (pool, edible and marsh frogs) and three brown frog species (common, agile and moor frogs). A–D and I were variants found only in the brown frogs, while E-H were variants found among the water frogs (Table 1.).

al. suggested that water frogs display the pattern shown here as E, and this was very much the case with 56 out of 66 (85%) individuals examined in agreement. No brown frogs had pattern E.

Patterns B-D were variations of the typical brown frog pattern A. F and G appeared to be variants of the more common water frog pattern E (all showed a shorter, broken posterior section with a somewhat different orientation to that of the main dorsolateral fold line). Fold pattern I (found

Fold pattern	А	В	С	D	E	F	G	Н	Ι	Total
Brown frogs	30	9	7	2	0	0	0	0	3	51
Water frogs	0	0	0	0	56	6	2	2	0	66
		D 1	1011							

 Table 1. Dorsolateral fold patterns (Fig. 5) observed in brown and water frogs.

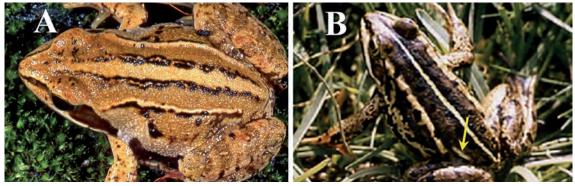


Figure 6. Examples of typical dorsolateral fold patterns. A = moor frog exhibiting typical brown frog dorsolateral fold pattern A (Fig. 5). B = pool frog (one of the last British native females, early 1990s) with a short "misaligned" posterior section (indicated by arrow) typical of water frogs.

in three brown frogs) appeared most like a broken variant of A-D (particularly A); the "chained" segments in the posterior half follow the general curving linearity of the folds (unlike the water frog pattern where the lower two or three "links" in the "chain" have a different orientation).

H(only found in two marsh frogs) was somewhat equivocal – but most resembled a broken variant of G. To help determine whether H was closer to E, F or I, it was of assistance to draw a line through the lower two "chain" segments of E-I and note that in H the lower two segments have a different linear direction to the rest of the curving form of the folds making it more consistent with the water frog pattern.

No patterns were common to both groups, however, as the sample number grew and more individuals with a chained pattern presented themselves, the dividing line became more tenuous to the point where, given more samples, this method may be best seen as a good generalisation. Photographs of frogs bearing typical dorsolateral folds are given in Fig. 6.

Presence/Absence of a Temporal Mask

The presence of a temporal stripe varied both within and between species. The results of the analysis of the 398 water frogs examined are given in Table 2. A small number of brown frogs completely lacked the temporal stripe (e.g. Fig. 7). This was more common in males than females and is particularly prevalent during the breeding season.

Further, temporal stripes were found in 33.6% of adult water frogs (4.1% of males and 63% of females) and 61% of juveniles. There is considerable variation within this trend: in northern

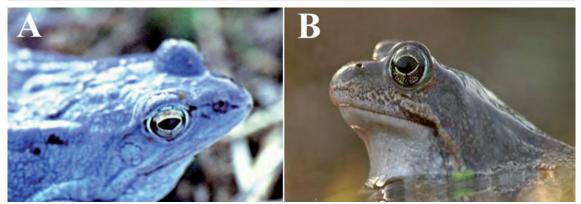


Figure 7. Brown frogs without a facial mask. A = a male moor frog from Sweden in breeding condition (Courtesy of Sven-Åke Berglind). B = breeding male common frog.

	PL Continental		PL N Clade		P. esculentus		
Mask	present absent		present absent		present absent		
Males	4	19	0	38	2	24	
Females	14	14 6		0	37	19	
Juveniles	15	2	29	0	17	4	
% adults with mask	4	1.9	53	3.7	47	.6	
% males with mask	1′	7.4	0)	7.7	7	
% females with mask	7	0	10	100		66.1	
% juveniles with mask	s 8	8.2	10	00	81		
	P. ridibundus			P. bergeri			
	P. ridil	bundus	<i>P. be</i>	ergeri	Р. ре	erezi	
Mask	<i>P. ridil</i> present			e rgeri absent	P. pe present		
Mask Males				0	-		
	present	absent	present	0	present	absent	
Males	present 0	absent 32	present 0	0	present 0	absent 6	
Males Females	present 0 4 0	absent 32 41	present 0 6 3	0	present 0 5 0	absent 6	
Males Females Juveniles	present 0 4 0 5	absent 32 41 11	present 0 6 3	absent 7 0 0	present 0 5 0 26	absent 6 8 1	
Males Females Juveniles % adults with mask	present 0 4 0 5	absent 32 41 11 5.2	present 0 6 3 4	absent 7 0 0 6.2	present 0 5 0 26	absent 6 8 1 5.3	

Table 2. Proportions of water frogs with and without a facial mask. Total number of frogs = 398(316 adult, 82 juvenile). KEY: P = Pelophylax, L = lessonae, N = northern.

clade pool frogs and *P. lessonae bergeri* 100% of the females and juveniles have a temporal mask but in *P. ridibundus* these figures are 9% and 0% respectively. Based on the sample examined here, a frequency ranking of the temporal mask is: northern clade *P. lessonae* and *P. l. bergeri* (although note the small sample number for *P. l. bergeri*) joint highest, followed by other European *P. lessonae*, *P.* kl. esculentus, *P. perezi* and *P. ridibundus*.

DISCUSSION

The results presented here, evaluating the methods for separating the northwest European brown and water frog groups, strongly validate the use of:

- 1) Relative distance between the eyes. The eyes of water frogs are closer together than those of brown frogs.
- 2) Inclination of the eyes. The eyes of water frogs are more upward looking than those of brown frogs.
- 3) Configuration of the dorsolateral folds.

The results show that separating the two groups on the basis of the presence/absence of a temporal mask is unreliable. Curiously, this was found to be the most frequent, long-standing and widespread method given in herpetofaunal literature.

Female and juvenile pool frogs *P. lessonae* from mainland Europe often have a temporal mask and

this was also the case in the female and juvenile edible frogs examined (Table 2). The temporal mask in the northern clade pool frogs of Norway and Sweden seems to be the norm as it appears to have been, from the remaining photographs and illustrations, in the now extinct British northern clade population. Identification guidance in literature, started in the 1800s, suggesting that the presence of a temporal mask indicated a brown frog species, could have led to under-reporting of British pool frogs, which, with the exception of breeding males, had a noticeable temporal mask and, as an added complication, were also brown rather than green. Fig. 8 shows examples of brown northern clade individuals with an obvious temporal mask.

Table 2 suggests that, the result of any random sampling would show greater variation in mask frequency in mainland European *P. lessonae* populations compared to the northern clade. The frequency of mask presence in the northern clade was: males 0%, females 100%, juveniles 100%. Whereas, in continental pool frogs the frequency was, males 17%, females 70% and juveniles 88%. The facial mask characteristic is widespread in Europe but, excluding the northern clade, seems to be particularly prevalent in pool and edible frog populations east of the Alps and in northern Italy. Handbook descriptions of water frogs lacking dark temporal markings were most accurate for adult



Figure 8. Northern clade pool frogs with a temporal mask. A = juvenile male from Norfolk (John Buckley). B = juvenile from Sweden (Jim Foster).

breeding males. In the case of the marsh frog the presence of a temporal mask is unusual but does occur occasionally in females (Table 2).

It is the author's experience that as male pool frogs mature, the facial mask becomes less distinct. In breeding males in nuptial colours there is no sign of the mask at all, though it can reappear, albeit faintly, from late summer to autumn, in some individuals. The water frogs most likely to be seen are the breeding males when positioned near the water's edge and advertising their presence with loud calls. It is perhaps this fact that has led to the mistaken impression that all water frogs lack the temporal mask.

Brown frogs, too, may lose their mask in the breeding season. Approximately half of the images from the Internet depicting breeding common frogs showed the males without a mask, in some instances the females too, and this condition was even more prevalent in breeding male moor frogs. It is evident that brown and water frogs cannot be reliably separated on the criterion of the presence or absence of a temporal mask.

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Influence of climatic gradient on spermatogenesis timing of *Trapelus lessonae* (Sauria, Agamidae) in the Zagros Mountains, Iran

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ABSTRACT - Spermatogenesis is a complicated process with various factors that influence and control it. I collected a number of male specimens of *Trapelus lessonae* in three latitudes (during biological activity) that were different in climate. I removed testes for histological survey. H and E staining techniques were used. The results of screening showed three phases of spermatogenesis during biological activity for three different latitudes. Spermatogenesis timing differed in the three latitudes. Timing of spermatogenesis differed in low elevation populations and began earlier than in higher elevation populations.

S PERMATOGENESIS is a complicated process with a range of factors that influence it. Molecular research indicates that spermatogenesis, and its associated programme for controlling gene expression, commences during changes from the germinal layer into spermatozoid (De Kretser, 1993; Sarge et al., 1995) and these influences vary across taxa (Phillips et al., 1987). Factors that affect the process can be divided into two groups - exogenous and endogenous. Both of these have a direct and indirect affect on spermatogenesis (Culler & Culler, 1977; Duvall et al., 1982; Licht, 1984). Examples of exogenous factors that can be named as environmental factors include temperature (Bona-Gallo & Licht, 1983; Gavaud, 1991), photoperiod (Mendoca & Licht, 1986; Whittier et al., 1987; Beaupre et al., 1997), dryness and moisture of environment (Shine, 1985; Vitt & Congdon, 1978). Examples of endogenous effects include steroidal hormones, nervous system (e.g., Tokarz et al., 1998; Rhen & Crews, 2002) and fat resource (Diaz et al., 1994; Castilla & Bauwens, 1990; Torki, 2006, 2007a,b). There are likely to be other factors that also affect spermatogenesis that are still to be discovered (Phillips et al., 1987).

In temperate-zone lizards with reproductive cycles influenced by climatic season, the male testicular cycle is divided into two well-defined phases: (a) the regenerative phase that occurs in the spring and is characterised by sustained sperm production, and (b) the degenerative phase that begins in late summer, where a break in spermatogenesis is observed (Fitch, 1970; Lofts, 1987; Castilla & Bauwens, 1990). Many studies have shown the effects of latitude on the reproductive ecology of animals (Rising, 1987; Young, 1994; Armbruster et al., 2001).

This study investigated the influence of climate gradient as an exogenous factor on spermatogenesis of *Trapelus lessonae* (Sauria, Agamidae) from the western Iranian plateau, on the western slope of the Zagros Mountains. The study site was located in Lorestan province between mid-Zagros (northern and eastern Lorestan) and southern Zagros (southern Lorestan).

Based on its climate Lorestan province is aptly named 'little Iran' (Torki, 2010), and has three different climates across three latitudes. These climates are as follows: (1) cool-temperate in northern Lorestan, geographic position approximately 34°05'N, 47°55'E, 1900 m ASL, 466.4 mm annual rainfall, (2) temperate climate in mid-Lorestan, geographic position approximately 33°29'N, 48°22'E, 1100 m ASL, 363.5 mm annual rainfall, (3) warm temperate in southern Lorestan, geographic position approximately 33°13'N, 47°49'E, 650 m and 337.4 mm annual rainfall.

Based on personal observations, biological activity of lizards in these three latitudes was crudely divided as follows; in the northern latitude, lizards hibernate for five months, from October to February (Torki, 2006, 2007b), in the mid-latitude, lizards hibernate for three months, from November to January, and finally in the southern latitude, lizards hibernate for two months, from December to January.

MATERIALS AND METHODS

Male specimens of *Trapelus lessonae* were collected across all aforementioned latitudes during normal diurnal activity. In the first latitude, 30 specimens were caught; in the second latitude, 33 specimens, and third latitude 42 specimens. Lizards were euthanized and testes of each specimen were removed during each month. Testes were fixed in 96% ethanol, cleared in xylene and embedded in paraffin. Histological sections were cut at 5-7 μ m, in haematoxylin followed by an eosin counterstain (H&E). The sections were then examined under light microscopy. To determine spermatogenesis timing and effects of geographic variation, a Tukey HSD test was used.

RESULTS AND DISCUSSION

Based on Tukey HSD test ($\alpha = 0.05$), spermatogenesis timing for the three latitudes varied as follows: Latitude (1) was divided into three phases (a) from March to May, phase (b) during June and July, phase (c) during August and September. Latitude (2) was divided into three phases, (a) from February to April, phase (b) during May and June, and phase (c) from July to October. Finally, latitude 3 was divided into three phases, (a) from February to April, phase (b) during June, and phase (c) from July to November. In all latitudes, during phase (a) spermatozoa were found in the lumen of seminiferous tubules with primary and secondary spermatocytes (Fig. 1). During phase (b), in most specimens, spermatozoa were found in the lumen of seminiferous tubules with primary and secondary spermatocytes. Finally, during phase (c) lumen of seminiferous tubules were found, but without spermatozoa or spermatocytes.

Usually spermatogenesis timing is related to environmental conditions (Duvall et al., 1982; Whittier et al., 1987). Timing of spermatogenesis activity has previously been described by the author (Torki, 2006, 2007 a, b). In this experiment, spermatozoa in the lumen of seminiferous tubules have been found. Therefore, spermatogenesis activity in this study occurred primarily during phase (a) because during this phase spermatozoa were found in the lumen of seminiferous tubules and also on primary and secondary spermatocytes. Based on these results, active spermatogenesis in the three latitudes occurred during two different times as follows: during March to May in the first latitude and during February to April in the second and third latitudes. Phase (b), named by the author as a 'transitional phase' (Torki, 2006, 2007a, b), had a duration of two months in the first and second latitudes but only one month in the third latitude. Inactive phases during biological activity in the first latitude were shorter (two months) than in the other latitudes (five months).

Spermatogenesis occurs in reptiles at different times (Sherbrooke, 1975; Torki, 2006, 2007a, b) across temperate regions. In reptiles, it is often observed in spring and early summer (Fitch, 1970; Lofts, 1987; Castilla & Bauwens, 1990). In some temperate regions, spermatogenesis in lizards occurs after hibernation (Fitch, 1970; Lofts, 1987; Castilla & Bauwens, 1990). In the tropics, spermatogenesis can occur continuously (especially in the ITCZ region = Inter Tropical Convergence Zone) (e.g., Sherbrooke, 1975; Hernandez-Gallegos et al., 2002; Torki, 2006, 2007a). However, in some tropical regions, spermatogenesis is not continuous and alternation of spermatogenesis occurs (Wilhoft & Reiter, 1965; Marion & Sexton, 1971). The effects of elevation in spermatogenesis timing in one tropical region have been shown to be zero (Hernandez-Gallegos et al., 2002) but in this study of a temperate species spermatogenesis timing is clearly related to elevation of the sampled lizard; that in turn could be related to temperature and other climatic conditions. At low elevations that have high temperatures, spermatogenesis commenced earlier in populations of T. lessonae than at higher elevations which have lower temperatures.

Lizards of temperate regions in other studies presented two periods in spermatogenesis with a degeneration and regeneration period (e.g., Lofts, 1987; Castilla & Bauwens, 1990). In the Zagros Mountains the degeneration period in autumn and winter (or hibernation period) occurred and regeneration started during spring and lasted across summertime biological activity (Torki, 2006, 2007a, b). The duration of the two periods therefore suggests that spermatogenesis is related to climate condition and hibernation timing for a number of lizards in the region (e.g., Diaz et al., 1994; Huang, 1997; Torki, 2007b). The results herein confirm

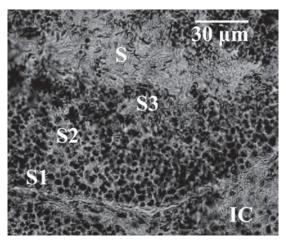


Figure 1. Seminiferous section during spermatogenesis active in phase (a) in *Trapelus lessonae*. S1: first Spermatocyte; S2: secondary Spermatocyte; S3: spermatid; S: Spermatozoa; IC: Interstitial cells.

this notion for Trapelus lessonae because duration of degeneration from cold climate (or first latitude) is shorter (seven months) than in the warmer, third climate (ten months). As winter temperatures in temperate regions are lower than spring months, some lizards undergo a hibernation phase where biological activities are dormant (Costanzo et al., 1995; 1988; Costanzo & Lee, 1995). Hibernation periods for poikilotherm lizards have an important role in survival because during hibernation lizards consume less energy and all of their remaining energy is stored for winter survival as well as for renewing the reproductive system (Costanzo et al., 1988; Storey et al., 1988; Grenot et al., 1996). During hibernation, lizards' body fat reserves are the only source to renew the testis volume and production of spermatocytes (Castilla & Bauwens, 1990; Heideman, 1995; Sharma & Shanbhag, 1992; Wapstra & Swain, 2001). This period for lizards inhabiting warmer, temperate climates like the third latitude, is shorter (two months) than cool temperatures or first latitude (five months).

The entire reproductive system is renewed during hibernation periods, and as a result, hibernation period has been called regeneration period for hibernating lizards. This strategy is the result of natural selection during evolution in each hibernating taxon (Storey and Storey, 1985; Costanzo, 1985; Ultsch, 1989; Litzgus et al., 1999; Torki, 2006, 2007a, b). This study confirms the influence of climate gradient to spermatogenesis timing in *Trapelus lessonae*, in the western Iranian plateau.

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Captive husbandry and reproduction of the Madagascan tree boa Sanzinia madagascariensis (Duméril & Bibron, 1844)

ADAM RADOVANOVIC

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Superior of all species from the Boidae. Its distinguishing features include a large off-set head and colour ranging from dark to light green with large rhomboid markings that continue down the body. In some individuals these markings are heavily bordered with white (Henkel & Schmidt, 2000). Neonates are a reddish-brown for the first few months of life (Henkel & Schmidt, 2000).

The species is listed as vulnerable on the IUCN Red List and CITES appendix 1 (IUCN, 2011). S. madagascariensis can inhabit a range of habitats from dry and moist forests, to savanna grasslands and is distributed throughout northwestern, northern and eastern Madagascar (O'Shea, 2007). It has heat sensitive pits between the upper and lower labial scales which is a feature not shared with the other two Madagascan boa species (Mattison, 1998). Juveniles lead an arboreal life whilst adults are commonly found basking on the ground or in low branches (Henkel & Schmidt, 2000). Their diet comprises small mammals and birds (O'Shea, 2007). S. madagascariensis is ovoviviparous with four to sixteen young born after a gestation period of six to eight months (Ross & Marzec, 1990).

MANAGEMENT

Three *S. madagascariensis* were used for the breeding programme (one male/two females). The male was an 11-year-old wild caught specimen. Both females were eight-year-old first generation captive bred specimens. Female 1 (Fig. 1) was the larger specimen and weighed 2500 g, female 2 weighed 2100 g.

The snakes were housed individually and only introduced together for breeding purposes. Specimens were housed in large fibreglass vivariums measuring $120 \times 60 \times 60$ cm. Abundant branches were provided inside enclosures to facilitate climbing. A large basking area was also provided.

Ambient day temperature was 24 to 28°C with a basking area that reached up to 35°C. Ambient night temperature was 20 to 22°C. Humidity was maintained at 40 to 60% RH by spraying with warm water every two days. Specimens were fed on one adult rat every three to five weeks.

REPRODUCTION

The decision was made to breed the larger female (female 1) in year one (end of 2008-2009) and the smaller female (female 2) in year 2 (end of 2009-2010). Breeding behaviour was very similar in both females. Therefore the breeding observations from both years are presented together.

From November to February, night time temperatures were gradually lowered between 14 to 16°C over five days. On day six, the male was introduced to the female's enclosure and copulation commenced 30 minutes later. The male was observed using his spurs during every introduction.

Copulation was observed mainly in the morning from 8:00 to 11:00 when body temperatures were between 16 and 18°C. Copulation was sporadic throughout November and the male was removed. All specimens refused food after their first introduction. The male was then reintroduced in December when female behaviour became constant in activity and thermoregulation. The male was deliberately introduced when it showed increased rapid tongue flicking. Copulation was frequently observed for a few days after reintroduction. After a week together, copulation was induced by spraying the enclosure and the specimens with warm water. When mating behaviour and copulation ceased, the male was removed. This method was continued from December through January. Copulation was observed on sixteen separate occasions with female 1 and on six occasions with female 2.

Ovulation in both females could not be observed but continued periods of basking were, from mid-March (2009) in female 1 and the beginning of March (2010) in female 2. Basking occurred every morning and usually lasted all day in both specimens. Female 1 raised her body temperature to 38°C by the afternoon whilst female 2 sought shelter if her body temperature had risen above 33°C. Temperatures were taken using an infrared heat gun. Both females began to darken in colour after their first slough to retain body heat for longer to bring on the developing ova (Ross & Marzec, 1990). This continued through to parturition.

Female 1 sloughed almost two months prior to giving birth and female 2 was in slough whilst giving birth. The day prior to parturition, female 1 was offered, and consumed, one large rat whereas female 2 refused food until her post parturition slough. Female 1 gave birth to three live neonates at the end of August weighing 52 to 56 g and six infertile ova. Female 2 gave birth to five live neonates (Fig. 2) at the end of August weighing 42 to 47 g, three still-born weighing 25 to 44 g and one infertile ova. Both specimens returned to normal colours after a post parturition slough.

REARING NEONATES

All three neonates from female 1 were housed individually in contico boxes on a rack system measuring 37 x 25 x 13 cm (L x W x H). Bark chippings and sphagnum moss were used as substrate and small sticks were used to provide climbing opportunities. Neonates were offered one small thawed mouse each. For the first two months specimens struck at food items but released and did not eat. After this period freshly killed mice were offered and all three specimens accepted. Eventually all three were weaned on to thawed mice after four months. All five neonates from female 2 were housed individually in plastic Hagen tanks measuring 27 x 16 x 20 cm. The enclosures were furnished using the same method as the neonates from female 1. All neonates accepted thawed, small mice after a month from birth.

Humidity lower than 40% RH resulted in dry sloughs and neonates had to be submerged in warm water for a few hours for the skin to be manually removed. Humidity was generally kept above 50% RH and sphagnum moss piles were always damp. Neonates were kept between 25 to 30°C.

After approximately four sloughs, and over six to eight months, the juvenile boas began their ontogenetic colour change from a red/brown to a light/dark green background (Fig. 3 and 4).

DISCUSSION

Sanzinia madagascariensis has been kept at the Birmingham Nature Centre for over 15 years. Specimens have included wild caught and captive bred individuals. Various methods for breeding have been tried over the years with the three specimens used in this breeding programme but with no success. The first successful breeding occurred using the above method. This was later replicated using a different female confirming the factors necessary for successful reproduction.

From the observations made herein, *S. madagascariensis* copulates readily in captivity. This was observed more frequently in female 1 and could possibly be caused by compatibility between individuals although in both cases, fertile mating took place.

S. madagascariensis seemed to be able to withstand lower temperatures during the cycling period than other boa species, without becoming susceptible to respiratory infections (pers. obs.). Keeping *S. madagascariensis* at temperatures as low as 14°C for short periods of time may aid fertility in the species (Ross & Marzec, 1990).

In previous breeding attempts, specimens were introduced only at the end of the temperature cycling period. Introducing the sexes at the beginning and throughout the cycling period may be beneficial in allowing the male to mate during the onset of ovulation. *Sanzinia madagascariensis* will mate throughout the year if introduced together under the correct conditions (pers. obs). However, successful reproduction appears to occur only with temperature fluctuations from November and neonates being born in August of the following year. Youll (2007) observed similar breeding success during these months.



Figure 1. Green adult female Sanzinia madagascariensis.

Figure 3. *Sanzinia madagascariensis* neonate showing red/orange coloration.



Figure 2. Sanzinia madagascariensis neonates.

During gestation, *S. madagascariensis* basks continually, however, if the basking temperature is not appropriate (between 30 to 38°C in this study) it may cause the developing ova to be re-absorbed (Ross & Marzec, 1990). Whilst writing this paper (July 2011) female 1 gave birth again nearly two years after her first breeding success using the same method for breeding.

Further studies of future breeding of *S. madagascariensis* in larger numbers may help to define whether the method for breeding used in this study can be successfully replicated in other collections. Captive breeding of snakes often needs to be performed more than once to allow accurate analysis of results and to determine factors that may or may not affect reproduction.

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Figure 4. Juvenile *Sanzinia madagascariensis* after ontogenetic colour change at approximately eight months from birth. Specimen is showing adult coloration.

NATURAL HISTORY NOTES

CORALLUS HORTULANUS (Amazon tree boa) and LEPTODEIRA ANNULATA (banded cateyed snake): HABITAT. The boid snake Corallus hortulanus (Linnaeus, 1758) is widespread across Central and South America, ranging from southwestern Costa Rica, Panama, northern South America (east Andes), Venezuela, Guiana, Amazonian Colombia, Ecuador, Peru, Bolivia and Brazil. It is also known from wet forests in southeastern Brazil and islands off Venezuela. Trinidad and Tobago, St. Vincent, Grenada, and Panama (Henderson, 1993). Henderson (1997) reports two subspecies of C. hortulanus; C. h. hortulanus (from Guiana, Amazonia and Brazil) and C. h. cooki (from Central America, Colombia, Venezuela, Trinidad, Tobago, St. Vincent and Grenada). The dipsadid snake Leptodeira annulata (Linnaeus, 1758) is widely distributed across the neotropics, ranging from the Amazon Basin of South America (Ecuador, Peru and Bolivia) to the Atlantic coast of Brazil (Duellman, 1958; Peters & Orejas-Miranda, 1970). According to Vrcibradic et al. (1999), most of the published information on the ecology of L. annulata in South America originates from Amazonian populations.

Herein we report the occurrence of Corallus hortulanus and Leptodeira annulata at Restinga de Iquiparí (21º44'S, 41º01'W; at sea level), within the municipality of São João da Barra, Rio de Janeiro state, southeastern Brazil. The restinga is located next to the delta of Paraíba do Sul River, in a lagoon complex that measures ca. 4800 ha. Some authors have noted that this area harbours high ecological diversity (Lamêgo, 1946; Suguio & Tesler, 1984). During nocturnal fieldwork, we collected a specimen of Corallus hortulanus (Museu Nacional do Rio de Janeiro, MNRJ 20065 [Fig. 1]) on 09 November 2010 and a Leptodeira annulata (MNRJ 20396 [Fig. 2]) on 09 April 2011. Despite both species' morphological adaptations to an arboreal existence (enlarged vertebral and paravertebral scale rows and a laterally compressed body [Duellman, 1958]), both snakes were found on the ground, perhaps during migratory behaviour.

Corallus hortulanus has been reported from evergreen wet and rain forests, banana plantations, mangroves and fruit orchards (Henderson, 1993).

Despite its widespread geographic distribution, this is the first time that *C. hortulanus* has been recorded in a restinga habitat. We are unaware of the occurrence of *Leptodeira annulata* in open areas and restinga habitats. Thus, our findings represent the first record of these species in restinga habitat.



Figure 1. Corallus hortulanus (MNRJ 20065). Photo by Caio A. Figueiredo-de-Andrade.



Figure 2. *Leptodeira annulata* (MNRJ 20396). Photo by Carlos Alberto Pereira Junior.

We thank Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis (IBAMA) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for capture permits and Museu Nacional do Rio de Janeiro (MNRJ) for housing the voucher specimens.

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CORONELLA AUSTRIACA Laurenti (smooth snake): GRAVID OVERWINTERING. The smooth snake *Coronella austriaca* is a small, nonvenomous snake that reaches the northwestern edge of its range in countries such as Norway and England. In the latter, its distribution is almost entirely restricted to lowland heath. Its secretive nature continues to hinder the understanding of even basic details of its behaviour.

An observation was made in April 2011 in Dorset, southern England, of a female smooth snake already showing signs of advanced gestation, namely; clear demarcation of the tail from the posterior part of the body, increased girth of the posterior two-thirds of the body (rather than a prey bulge) as well as a 'falling away' of the body from the backbone, which is associated with depletion of fat reserves in snakes during gestation (Fig. 1).



Figure 1. Female smooth snake showing signs of being gravid, April 2011. Note the clear distinction of the tail from the posterior portion of body, increased girth of the posterior two-thirds of the body, and 'falling away' of the body from the spine due to depletion of fat reserves during gestation.

During the course of survey work the female was weighed and measured at the start of the season, and the ratio of her mass to length was found to be substantially higher than that of twelve other female snakes sampled early in the season, although two other females also shared similar (but less conclusive) signs of being gravid. Throughout the spring and summer, this female also showed high site fidelity (always being found under the same artificial refuge or within 1 m of it), characteristic behaviour of gravid animals. The girth also persisted, and slowly increased, confirming that this was indeed not due to the presence of an especially large prey item.

The indications of advanced gestation so early in the year suggest that mating had occurred in the previous spring, and that the animal in question had overwintered whilst gravid. The possibility that gravid snakes may retain embryos over winter was proposed by Spellerberg & Phelps (1977) although there are no records confirming its occurrence (Beebee & Griffiths, 2000). Autumn mating has been recorded in the wild, including in England (Braithwaite et al., 1989; Bull, 2010), and this phenomenon could explain females showing early signs of gestation the following spring, but would not account for advanced gestation, such as in the female that is the subject of this note.

Typically, following spring mating, birth occurs three to five months later in August or September (Beebee & Griffiths, 2000). Records of individual snakes breeding biennially have therefore generally been attributed to the breeding year being followed by a fallow (non-breeding) year, presumably allowing females to build up reserves for the following season. Gravid overwintering, delaying birth until the year after mating, could also produce a pattern of biennial reproduction.

The current observation raises questions of whether overwintering in this state is common in England or elsewhere in the northern part of the species' range in Europe and whether it may also occur in the adder Vipera berus, which also gestates its young internally rather than laying eggs, and also has a biennial pattern of reproduction. Further work would be needed to answer these questions, including the possible use of X-ray or ultrasound examination to confirm gravid status, and the use of data from elsewhere in England and the species' northern (e.g. Norway) and core (e.g. central France, Italy) range, to allow comparison. Similar data could also be collected for adders. It has been reported that adders show some degree of true viviparity, i.e. that there is some direct transfer of nutrition from mother to young during their development within the female, but that smooth snakes are ovoviviparous, i.e. the young are entirely enclosed within egg membranes during development and presumably therefore receive less nutrition than adder embryos (e.g. Beebee & Griffiths, 2000). If transfer of nutrition to adder embryos is more efficient than in smooth snakes, it could be suggested as contributing to the observed difference in the species' northern limits.

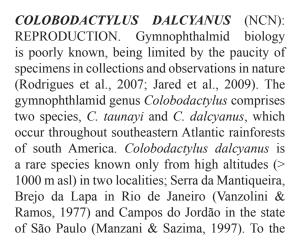
A more thorough study involving a larger sample of biometric data is planned for the next season, but in the meantime the author would be pleased to receive any observations regarding the possibility of gravid overwintering in smooth snake or adder, whether in support or against.

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best of our knowledge there is no information on the biology of *C. dalcyanus*.

This note provides the first observations of *C. dalcyanus* reproduction. Notes were taken during a herpetological survey of Campos do Jordão State Park during spring, 15-18 October 2005. Additional observations were made in a laboratory. Specimens were obtained 1940 m asl in a forest near a small stream surrounded by "Campus Montanus" environment (IBGE, 1992) (22°43'01.3"S; 45°27'43.3"W). The climate was 18°C and 55% RH.



Figure 1. Colobodactylus dalcyanus female (MZUSP 95598).

Four female C. dalcyanus with eggs in their oviducts were collected during this survey. Two of them were preserved (MZUSP 95601, 95602) while the remaining two specimens (MZUSP 95598, 95603) were kept alive and transferred to the laboratory where they laid eggs. All four specimens retained two eggs, one in each oviduct, fitting the clutch size pattern of two eggs recorded for most Gymnophthalmidae (Pianka & Vitt, 2003). One female (MZUSP 95598) (Fig. 1) was found under leaf litter, curled around its laid eggs. This female did not show any defensive behaviour. When disturbed it reacted by moving the body without loosening the curl around the eggs and remained inactive when left in-situ. A second female (MZUSP 95603) was collected by pitfall trap from the same forest area and laid two eggs in a plastic container. This specimen was transferred with the eggs to a terrarium covered with the litter vegetation from its capture area. After approximately one hour the female curled around the eggs and kept this position for 30 hours.

These two records suggest initial parental care of eggs by *C. dalcyanus*. Parental care is rare in

reptiles and possibly evolved independently in several lineages (Shine, 1988; Greene et al., 2006). The behaviour described herein for *C. dalcyanus* has also been observed for *Leposoma puk* (M. Dixo, pers. comm.). This suggests that parental care may be more common among gymnophthalmids than expected.

The two eggs laid in captivity were subsequently fixed at different day intervals to provide embryological data. Developmental stages of the embryos were established by an approximation with the developmental table for

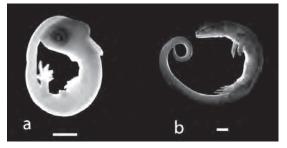


Figure 2. Embryos of *Colobodactylus dalcyanus* from female MZUSP 95603. a) Embryo MZUSP 99608 at stage 36 (Dufare & Hubert, 1961). b) Embryo MZUSP 99609 at stage 40. Scale bar = 1.0 mm.

Lacerta vivipara (Dufaure & Hubert, 1961). The first egg was opened 32 days after oviposition and revealed an embryo (MZUSP 99608; SVL= 12,6 mm) in stage 36. Digits were already differentiated but the interdigital membrane was still in the process of being absorbed (Fig. 2a). The second egg was opened after 56 days and revealed an embryo (MZUSP 99609; SVL = 26.7 mm) in stage 40 (Fig. 2b). According to Dufare & Hubert (1961), stage 40 is one of the latest stages before hatchling, being characterised by pigmented scales, closed parietal fontanel and the presence of an egg tooth. The presence of an embryo with 56 days pre-hatchling morphology indicates a period of embryological development of approximately 60 days, under controlled conditions.

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ODONTOPHRYNUS CARVALHOI (Carvalho's escuerzo): MALFORMATION. Amphibians' permeable skin, poorly protected eggs and embryos, and biphasic life cycle make them particularly sensitive to environmental change. The occurrence of malformations in a few individuals is expected in healthy populations and may be related to natural mutations, developmental errors or predation (Blaustein & Johnson, 2003). Trematoda parasites, UV radiation, environmental pollutants, and/or the synergism between these variables may also increase abnormalities among natural populations (Loeffler et al., 2001; Kiesecker, 2002; Ankley et al., 2004; Burton et al., 2008). The commonest types of malformation in amphibians are the absence of limbs or the presence of extra ones (Ankley et al., 2004; Meteyer, 2000). Abnormalities in vital organs are less common and drastically reduce chances of survival during the larval period (Loeffler et al., 2001).

In this note, we report a case of anophthalmia in Odontophrynus carvalhoi Savage and Cei, 1965, an anuran species found in rainforests of eastern Brazil. Observations took place at Parque das Trilhas, municipality of Guaramiranga, state of Ceará, northeast Brazil (04º16'S, 38º56'W; 880 m asl). The area comprised 70 ha of conserved tropical rainforest that was continuous with surrounding 1,584,836 ha of forest within an area of environment protection under the State's responsibility. On 10 April 2009 at 11:15 an O. carvalhoi (SVL 64.18 mm; 34 g) was found dead near a small stream inside a conserved forested area. Upon inspection we found that the anuran's left eve was missing. There was a lack of scars or sign of injury, suggesting this was a case of

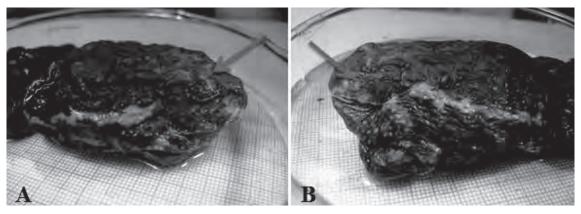


Figure 1. *Odontophrynus carvalhoi* with anophthalmia found in Guaramiranga, Ceará. A – Right side with arrow pointing to closed eye; B – Left side with arrow pointing to where eye should be.

anophthalmia (sensu Meteyer, 2000) (Fig. 1). McCallum & Trauth (2003) after analyzing 1,464 Acris crepitans, found that 104 of them presented malformations, with only 1% of these being anophthalmia. A similar proportion of malformed individuals with missing eyes was reported by Quellet et al. (1997) in a study of four anuran species from Canada. Cases of anophthalmia in anurans have been attributed to the presence of pesticides, UV-light and viruses (Quellet et al., 1997; Blaustein & Johnson, 2003; Burton et al., 2008). The area where the observation took place has been regularly visited by the authors (LBMB and FAA) and from 500 individuals (11 species), only two others (Leptodactylus gr. pustulatus and L. vastus) presented some type of malformation. Both these were limb related. Among total numbers of O. carvalhoi found this single case of anophthalmia represents 1.85% of all individuals (1/54). This low incidence of deformities indicates that the case is probably natural (Blaustein & Johnson, 2003).

To the best of our knowledge this is the first report of malformation in the genus *Odontophrynus*. Monitoring cases of malformation may help better understand the dynamics of abnormalities in the species and could be useful in the evaluation of the environmental health in the area. It is also important to monitor new incidents of malformation as some cases can expand as in the United States, where some 54 species have been registered with malformations in 44 states. Some of these areas have as many as as 80% of the individuals with some form of abnormality (Blaustein & Johnson, 2003; McCallum & Trauth, 2003; Schoff et al., 2003). We hope that this report will encourage other researchers working in the region and in other developing countries to monitor and publish such findings. This would assist mapping of occurrences of malformations in amphibians globally.

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RHINELLA JIMI (cururu toad): PREDATION. Several studies show that anurans help maintain energy flow in biological systems by being prey items (Ranvestel et al., 2004; Toledo, 2005; Altig et al., 2007; Toledo et al., 2007). *Rhinella jimi* (Stevaux, 2002) is a Bufonid distributed throughout Atlantic Forest and Caatinga in northeast Brazil (Frost, 2010). It belongs to the *Rhinella marina* group, distributed throughout south America (Maciel et al., 2010). The toads are easily identified because of broad parotoid glands used in defence (Wells, 2007). Ingestion of its bufotoxin may cause tremors, paralysis, convulsion and even death in predators (Fearn, 2003; Sonne et al., 2008; Jared et al., 2009).

Athene cunicularia (Molina, 1782) is a burrowing owl of the Strigidae and is widely distributed throughout the Americas (Korfanta et al., 2005; Salazar, 2007). Its diet includes small vertebrates and invertebrates (Tyler, 1983; Martins & Egler, 1990; Wiley, 1998; York et al., 2002; Motta-Júnior, 2006).

On 23 September 2010, we witnessed a predation attempt on a R. jimi by A. cunicularia. The observations occurred at Emendadas Village, Poco Redondo, Sergipe State, northeastern Brazil (09°48'34.1"'S, 037°41'20.4"'W; 198 m asl), Caatinga biome. We witnessed three attacks between 19:00 and 22:00. On two occasions, the bird flew to other perches carrying the anurans in their claws. On one occasion at 20:20 the owl ran away across the ground and left the R. *iimi* (SVL 115.09 mm). The attacks were performed mostly with the claws followed by pecks to the dorsal region and head. The toad was collected and housed in the Universidade Federal da Paraíba (CHUFPB 00105).

Occurences of predation on *Rhinella jimi* are scarce in literature and this rarity of documented predation possibly reflects its noxious toxicity to predators (Jared et al., 2009). Despite this there is a range of animals such as snakes, birds, mammals and invertebrates, including species of the *R. marina* group (Toledo, 2005; Toledo et al., 2007) that do consume toads containing bufotoxin.

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OXYBELIS FULGIDUS (green vine snake). DIET. Oxybelis fulgidus is an arboreal and diurnal snake with a distribution ranging from southern Mexico to northeastern Argentina. On 18 May 2011 at 15:26 one of us (ERV) observed an adult O. fulgidus capture and feed on a clay-coloured thrush Turdus grayi. The snake was perched at a height of 3 m in a Ficus colubrinae tree outside the offices of the pre-montane tropical forest of Tirimbina Biological Reserve, Heredia Province, Costa Rica. Shortly after (ERV) first noticed the snake, an adult T. grayi landed on the tree less than a metre away from the snake and within 20 seconds the snake successfully struck and captured it, whereupon the bird remained alive for approximately ten minutes (Fig. 1). The snake had difficulty ingesting the bird since the bird's shoulder width was wide relative to the snakes gape, however, the snake successfully consumed the bird and the entire feeding event lasted 2 hours and 45 minutes (Fig. 2). After fully consuming the bird, the snake descended from the tree and moved away. *O. fulgidus* is known to prey upon a wide variety of lizards and a variety of birds (Scartozzoni et al., 2009) but this observation marks the first documentation of *O. fulgidus* feeding on *T. grayi*. We thank Tirimbina Biological Reserve.

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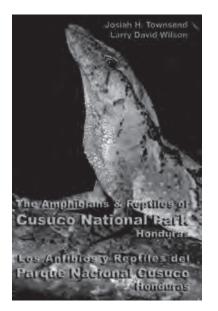
Figure 2. Oxybelis fulgidus consuming Turdus grayi, Tirumbina, Costa Rica.



Figure 1. Oxybelis fulgidus capturing Turdus grayi, Tirumbina, Costa Rica.

The Amphibians and Reptiles of Cusuco National Park Honduras.

Josiah H. Townsend and Larry David Wilson 2008, Bibliomania! Salt Lake City, Utah, 322 p.



Herpetofauna of Cusuco National Park, Honduras is one of the first field guides dedicated solely to the herpetofauna of a single protected area. It is a bilingual treatment of the herpetofauna of the national park with left-hand pages bearing the English text, and the right bearing the Spanish translation. But this is no mere guide to herp species; it is a guide to the National Park as well. Subjects covered include a description of the various habitats found within the 234 km² of the park (from dry mesic to cloud and elfin forests), directions on how to get to the park, a guide to the six trails that spread out form the visitor centre and a description of the camping facilities at the visitor centre and more remote campsites found inside the park and how to get to them.

The majority of the book is of course taken up with the species descriptions. These are complete with the obligatory keys at the beginning of each section complete with very useful, and superbly drawn, line drawings and photos to describe various important diagnostic features, as well as a nice colour coded diagram to the scalation of colubrid snakes.

Then the first cracks start to appear from the very first species account. Firstly, the font used for the species name was not the easiest to read. Also throughout the book the photographs are lacking legends. This was more than a little inconvenience, particularly in the species accounts. It does not matter too much if there is only one photograph included in the account as they are embedded within that species account. Having the photographs with the relevant species account is very useful as it means one does not spend lots of time flicking from the account to the photograph. However, if more than one photo appears it is not immediately clear if you are looking at different sexes, ages or colour morphs. An example of how this can get confusing is in the accounts of Stenorrhina degenhardtii and Tantilla schistosa where due to the way that the English and Spanish text run on opposing pages the photograph of the former appears in the Spanish text for that species but next to the English text for the latter.

This was a real let down for me as the information contained within the accounts is well written and inherently useful. Information included in the account includes common name, holotype locality, similar species, species description, distribution, the localities the species has been found within Cusuco National Park (this relates to the information given about the trails and gives a very good idea of where you might look for each species), natural history, conservation status, finished off with remarks and references.

After the species accounts follows a comprehensive treatment of the conservation status of Cusuco's herpetofauna covering trends such as levels of endemicity and the vulnerability of various elements of the herpetofaunal community to mention a few. All of its faults aside the detailed information contained within this book makes it an invaluable to anyone planning further research in the National Park. It will also be of interest to more casual herpetologists visiting either Honduras or the National Park itself.

ROLAND GRIFFIN

The Snakes of Honduras – Systematics, Distribution, and Conservation.

James R. McCranie

2011, Society for the Study of Amphibians and Reptiles, Thomson-Shore, Michigan. 724 pages.

At first glance one gets a sense of the enormity of this project, but it is not until you read the first main chapter entitled 'Materials and Methods' that you truly get a glimpse of the effort and time gone into preparing this volume. It is not only the synthesis of all known literature on Honduran serpents, but also the cumulative data from over 6000 specimens held in collections throughout the World, most of which were examined by the author himself. This sample constitutes the vast majority of all known specimens with a Honduran origin. The next two chapters include a succinct description of the Honduran environment, climate, biomes and a history of herpetofaunal work in the country.

Once one starts reading the species accounts, which rather unsurprisingly make up the bulk of this book, it becomes immediately obvious that they have been meticulously written. The accounts start with a key to the families of snakes present in Honduras in both English and Spanish, followed by the species accounts organised taxonomically. Each new genus is started by a bilingual key. The keys are usefully illustrated to show various diagnostic features. The species descriptions are very detailed and contain information on morphology and scalation, hemipenal structure, coloration in life and in alcohol, and a list of all the specimens examined. Additional information in each account includes, but is not limited to, changes in nomenclature, distribution, similar species, illustrations contained in other literature, and natural history. Additionally, maps showing the localities of where the specimens were collected are included at the end of each description, unless more than one species is illustrated whereupon the map appears after the first species described.

Following the species accounts are the now ubiquitous chapters on conservation issues. McCranie has done superb job of summarising issues particular to Honduras, many of which, though not all, will be familiar to workers throughout the world. These go some way in adding to the growing body of evidence showing how widespread difficulties faced by herpetofaunal communities really are. The book finishes with colour plates, glossary, gazetteer and literature sections all composed in the fashion followed in the rest of the book.

My main criticism of this book regards the small photographs in the colour plate section at the back of the book that make it quite difficult in some cases to distinguish some of the key identifying features or get a true feel for the species in question. Given the advances in digital photography this is a shame, but equally it is understandable as the inclusion of larger, and additional photos would most likely make this book unwieldy and very expensive to produce. Ultimately, given the quality of the work this down side can easily be forgiven.

McCranie and SSAR should be applauded for this magnificent volume. It is the second in a trilogy of works between *The Amphibians of Honduras* (McCranie & Wilson, 2002) and the eagerly anticipated second instalment of the reptilian fauna of Honduras (McCranie, in prep). Once completed it will be one of the most thorough treatments of the herpetofauna of a single country, currently rivalled only by Savage's 2002 epic treatment of the herpetofauna of Costa Rica (Savage, 2002).

The Snakes of Honduras is a wonderfully presented and marvellously detailed book and truly a must for anyone with an interest in the snakes of Middle America. It is definitely worth considering as a purchase by anyone with anything close to an interest in the wider subject of herpetology.

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