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Diet of Juvenile *Varanus niloticus* (Sauria: Varanidae) on the Black Volta River in Ghana

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Published accounts of the diet of *Varanus niloticus* suggest that it is a generalized feeder, eating a wide range of invertebrates and vertebrates that are caught by hunting and scavenging (reviewed by Losos and Greene, 1988). Examination of the skull has suggested an ontogenetic change in diet from a generalized feeder to one specialized for molluscivory (Rieppel and Labhardt, 1979), but there is no direct evidence of a high degree of molluscivory in this species.

Little is known about the diet of juvenile *V. niloticus*, nor of the juveniles of most other *Varanus* species. Schmidt (1919) examined nine juvenile *V. niloticus* collected in Zaire and reported that only three contained food (mantid, grasshopper, frog, four slugs, crickets and unidentified insects). Auffenberg (1994) examined the stomach contents of 92 juvenile *V. bengalensis* from various locations and found them to be entirely insectivorous, feeding mainly on hymenopterans and coleopterans. His study suggested animals from Pakistan accumulated large amounts of fat in the first few months of life preceding a period of fasting.

Varanus niloticus is heavily exploited throughout Africa for its meat and skin (Luxmoore et al., 1988), but there have been few ecological studies of the animal on which to base conservation strategies (Lenz, 1995). This study investigated the feeding ecology of young *V. niloticus* by examination of diets and fat bodies in a sample taken from a 12 km stretch of the Black Volta River. In this study "juveniles" are animals of the youngest age class (probably no more than four months old), and "adults" refers to all other age classes. Knowledge of the diet of juvenile monitor lizards is of particular interest because very little is known about the ecology of the juveniles of any *Varanus* species.

I report on the alimentary contents of 43 juvenile [mean SVL = 143 ± 2.7 mm (SE); mean mass 41 ± 2.4 g] and five adult (mean SVL 390, ± 32.7 mm; mean mass 1134 ± 373.8g) *V. niloticus*, caught on the Black Volta River between Batoo village (8°17'N, 2°15'W) and Bope camp (8°22'N, 2°17'W) between 9–29 July 1996, and deposited in the Cape Coast University Museum, Ghana. Animals were collected by canoe as they lay in riverbank vegetation. Alimentary tracts of preserved animals were opened and all items removed. Gut contents were rinsed into a strainer with mesh size of approximately 1 mm² and examined under low-power magnification. Identifiable fragments were recorded and identified to order. Prey were classified according to residence in the stomach or lower alimentary tract (referred to in this work as "lower gut"), depending on whether the bulk of the item lay proximal or distal to the pylorus, respectively. Recognizable fragments other than single limbs were recorded as prey items. Intact prey items were

TABLE 1. Diet of juvenile *Varanus niloticus* (N = 43), expressed as percentage of total number of prey items.

Prey taxa	Stomach	Lower gut
Spiders	9.8	16.7
Snails	2	6.9
Crabs	0	9.8
Larvae	6.9	8.8
Frogs	2.9	0
Orthopterans	7.8	15.7
Beetles	0	2.9
Miscellaneous	2.9	6.9
Millipedes	0	0
Fruit	0	0

blotted dry and weighed to the nearest 0.01 g. Visceral fat bodies were weighed to nearest 0.01 g. Means ± 1 SE are given.

Almost all animals had fragments of vegetation in the gut, presumably accidentally ingested, which were not analyzed further. The 43 juveniles contained a total of 104 prey items, 34 in stomachs and 70 in lower guts (Table 1). Twenty-five juveniles had empty stomachs, five had empty lower guts and one had no food items in the alimentary tract. For juveniles, mean number of prey was 2.4 ± 0.2, mean number of prey in stomach was 0.7 ± 0.2, and mean number of prey in lower gut was 1.6 ± 0.2. Mean mass of 18 intact prey items from preserved stomachs of juvenile *V. niloticus* was 0.36 ± 0.07 (range 0.03–0.9) g. Using mean juvenile mass (40 g) as predator mass gives mean predator/prey mass ratio of 0.009 ± 0.002 (range 0.001–0.023). There was no relationship between the time of day of capture and number of prey items in the stomachs ($R^2 = 0.018$) or lower guts ($R^2 = 0.086$) of juveniles. Five adults contained 20 prey items (mean = 4.0 ± 1.0, range 0–8). One adult had no food in the entire alimentary tract.

Spiders and orthopterans were the most common prey of juveniles, both occurring in 45% of lizards examined. Larvae (Coleoptera and Leiodoptera) occurred in 30% of the sample and snails in 21%. Although 23% of lower guts contained crabs, none were found in stomachs. Miscellaneous prey were unidentified or represented by a single prey item. Adults contained beetles, neem seeds (*Azadirachta indica* family Meliaceae), orthopterans, a millipede and a snail.

Eight juveniles contained fat bodies averaging 0.05 ± 0.03 g (range 0.01–0.22). There was no significant difference in the mass of juveniles with or without fat bodies (Mann Whitney *U*-Test: $W = 447$, $P = 0.76$).

With the exception of caterpillars and some spiders which occur along the riverbank, most prey items are probably found by active foraging within or under the herb layer of the forest. The supposition that juveniles dig for food is supported by the presence of nocturnal, burrowing, lycosid spiders in the diet. In fact many prey items (crabs, orthopterans, beetles and beetle larvae) could have been dug up or caught in burrows. There is no evidence that juveniles catch any food in the water, although it is possible that frogs and crabs are caught there and that other invertebrates are caught after they have fallen into water.

Auffenberg (1994) reported on a sample of 92 pre-

served juvenile *V. bengalensis* which contained an average of almost 12 items per lizard, with predator-prey mass ratios of 0.006:0.5. He also stated that body fat accounted for over 28% of body mass in juvenile *V. bengalensis* from Pakistan, where the animals do not feed for several months at the age of nine months. In contrast, juvenile *V. niloticus* contained only negligible amounts of body fat. Their relatively low rate of food intake is unlikely to be the result of local food shortages, because all specimens appeared in good condition. Lack of fat accumulation in these animals therefore suggests that they do not undergo extended fasting.

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Traumatic Injuries in Two Neotropical Frogs, *Dendrobates auratus* and *Physalaemus pustulosus*

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Traumatic injuries in amphibians have been attributed to unsuccessful predation events (Martof, 1956;

Dubois, 1979; Ouellet, 2000). Other possible sources of injury in amphibians are the result of fighting with conspecifics, mechanical accidents or scarring from infections (Dubois, 1979; Ouellet, 2000). Because most adult frogs do not have claws or teeth that could be used as weapons during combat, conspecific-inflicted injuries are unlikely. Mechanical accidents are possible in frogs, although the most likely source of such injuries are anthropogenic or acquired during the escape from predators. Scarring caused by infection is possible but has not been rigorously investigated as a source of injury in nature. The most likely source of traumatic injury is therefore through interactions with predators (Martof, 1956; Dubois, 1979). However, the majority of large-scale studies of traumatic injuries in anurans have been conducted in temperate areas (Rostand, 1949; Martof, 1956; Dubois, 1979; Ouellet, 2000). It is not known whether predation is the most likely source of traumatic injury in Neotropical anurans.

Before injury rates can be used to test hypotheses about predation, the nature of traumatic injuries and whether they reflect predation pressure in the Neotropics must be examined. If they do reflect predation, we would expect that species with differing levels of predation pressure would have correspondingly different levels of traumatic injury. To examine this, two sympatric species of similarly sized frog, the green poison frog, *Dendrobates auratus*, and the túngara frog, *Physalaemus pustulosus*, were surveyed for relative levels of traumatic injury. *Dendrobates auratus* is a diurnal terrestrial frog with toxic skin secretions containing many alkaloids (Daly and Myers, 1967; Daly et al., 1987). In contrast, *P. pustulosus* is cryptic and does not secrete alkaloids (Daly et al., 1987). Little is known of the predators of dendrobatid frogs in the wild. For *D. auratus*, predator accounts are based on single observations (a fish, *Brycon guatemalensis* in Hedstrom and Bolaños, 1986; a bird, *Baryphthengus marhi* in Master, 1998). The only well-documented predator of *D. auratus* is the theraphosid spider *Sericopelma rubronitens* (Summers, 1999), which is deterred by the frog's toxicity (Gray, 2000). In contrast, *P. pustulosus* is palatable and has a wide range of known predators (Ryan, 1985; Gray et al., 1999). In addition to differences in palatability, these two species have different reproductive strategies and differ in life span (Ryan, 1985; Summers, 1989, 1990). However, they are about the same size and are found syntopically in many of the same habitats. The Panamanian Island of Taboga is home to large populations of both species. On Isla Taboga, both *D. auratus* and *P. pustulosus* are active during the day, which contrasts with the primarily nocturnal behavior of all other studied populations of *P. pustulosus* (Jaegar and Hailman, 1981; Ryan, 1985). *Physalaemus pustulosus* were observed moving about the forest on Isla Taboga, calling and nesting throughout the day in full sunlight. Isla Taboga thus provides a perfect site to make comparisons because both species occur in high densities in the same areas and therefore are exposed to the same conditions. If there are trends in the injury levels that can be correlated with predation for these species from different anuran families, then traumatic injury may be used to understand predation pressure in a wide variety of frogs. This study represents the first population-level examination of traumatic injury in neotropical anurans.

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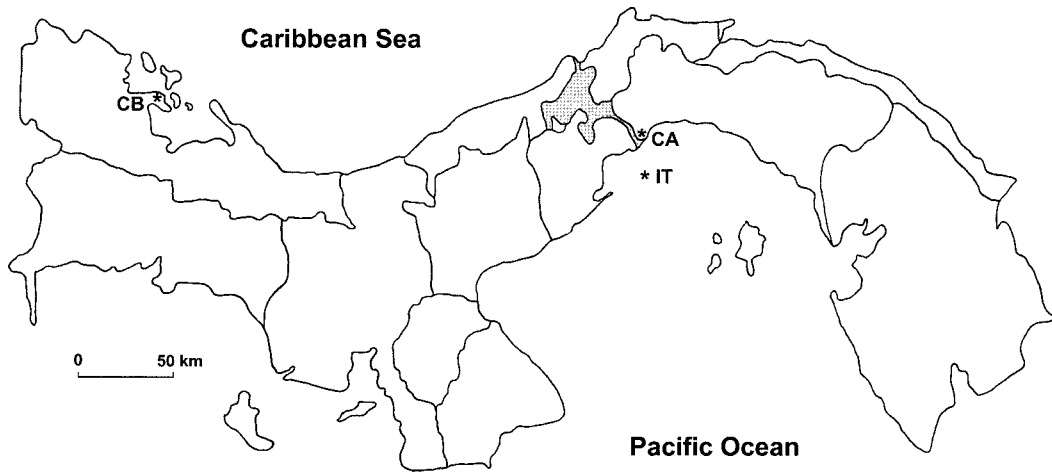


FIG. 1. Map of the Republic of Panama with sampling sites labeled (IT = Isla Taboga, CA = Cerro Ancon, CB = Cerro Bruja).

Study Sites.—Sampling was conducted in a forested nature reserve on Isla Taboga ($8^{\circ}47'N$, $79^{\circ}34'W$) in the Republic of Panama from April through June of 1998 and May through August 1999. To ensure that trends seen on Isla Taboga were not site-specific, sampling was also conducted at two additional sites, Cerro Ancon, a protected area in Panama City ($8^{\circ}56'N$, $79^{\circ}34'W$), and a cacao plantation on Cerro Bruja in Bocas del Toro ($9^{\circ}9'N$, $82^{\circ}14'W$; Fig. 1). Adult *D. auratus* were sampled both years at all three sites, whereas adult *P. pustulosus* were sampled only in 1999 and primarily on Isla Taboga. A small nocturnal population of *P. pustulosus* was sampled on Cerro Ancon to determine whether there were any differences between a nocturnal and a diurnal population. A total of 592 adult *D. auratus* were surveyed over the two years; 231 on Isla Taboga, 274 on Cerro Ancon and 87 on Cerro Bruja. A total of 230 adult *P. pustulosus* were examined; 188 on Isla Taboga and 42 on Cerro Ancon. Recently metamorphosed *D. auratus* ($N = 10$) and *P. pustulosus* ($N = 111$) were also surveyed on Isla Taboga in 1999.

Survey Methods.—Frogs were hand-caught and systematically examined for evidence of injuries. Injury

was visually differentiated from developmental abnormality by ascertaining the presence of scar tissue or a regenerative spike (Ouellet, 2000). All animals were measured (snout-vent length) to the nearest 0.1 mm using dial calipers and marked by clipping the terminal phalange of the fourth digit on the right hind limb. A sketch of the color pattern was made for each *D. auratus*, which, in conjunction with the clipped toe, allowed identification of recaptured animals.

Analytical Methods.—The sexes were considered together. Chi-square analyses were used to test for differences in injury occurrence within and between species (Zar, 1999). Snout-vent length data for the different species and sites were analyzed using one-way analyses of variance (SYSTAT vers. 9.0, SPSS, Inc., 1998).

High levels of traumatic injury were found in *D. auratus* (Table 1), the majority of which involved the partial or total amputation of digits (89.9%), but loss of forelimbs, hindlimbs, and eyes also occurred. For the three populations surveyed, Isla Taboga, Cerro Ancon, and Cerro Bruja, the rates of trauma were 14.7, 10.2, and 19.5%, respectively. There were no significant differences between rates of trauma in these pop-

TABLE 1. Number and type of traumatic injuries in Panamanian populations of adult *Dendrobates auratus* and *Physalaemus pustulosus*. Percentages are given in parentheses following the actual number of injuries encountered. (IT = Isla Taboga, CA = Cerro Ancon, CB = Cerro Bruja).

	<i>D. auratus</i>				<i>P. pustulosus</i>		
	IT	CA	CB	Total	IT	CA	Total
Number examined	231	274	87	592	188	42	230
Digit amputation	31	27	13	71 (12.0)	7	2	9 (3.9)
Limb amputation	—	1	1	2 (0.3)	—	—	—
Foot amputation	1	—	1	2 (0.3)	—	—	—
Hand amputation	—	—	2	2 (0.3)	—	—	—
Eye enucleation	1	—	—	1 (0.2)	1	—	1 (0.4)
Limb fracture	1	—	—	1 (0.2)	—	—	—
Total trauma	34 (14.7)	28 (10.2)	17 (19.5)	79 (13.3)	8 (4.3)	2 (4.8)	10 (4.3)
Age controlled				(4.4)			(4.3)

ulations ($N = 592$, χ^2 contingency test, $\chi^2 = 5.58$, $df = 2$, $0.05 < P < 0.10$), allowing the data to be combined to yield an overall trauma level of 13.3%. Eight *P. pustulosus* adults from Isla Taboga had traumatic injuries (4.3%), with the majority of injuries being to digits (87.5%). The level of traumatic injuries in the nocturnal Cerro Ancon population (4.8%; two adults with traumatic injury to digits) did not differ significantly from the Isla Taboga frogs ($N = 230$, χ^2 contingency test, $\chi^2 = 0.07$, $df = 1$, $0.75 < P < 0.90$) yielding a combined total injury level for *P. pustulosus* of 4.3%. When *P. pustulosus* from both sites were considered together, 90.0% of all their injuries were to digits.

When *D. auratus* that were originally captured in 1998 were recaptured in 1999, seven of 34 (20.6%) recaptured animals had new injuries. On Isla Taboga, three of the 20 recaptured animals had new injuries (15.0%), whereas four of 14 (28.6%) recaptured animals had new injuries on Cerro Ancon. Only Isla Taboga and Cerro Ancon populations were considered as these sites had the greatest sampling efforts with 19.7% of animals caught in 1998 recaptured in 1999. The rate of new injury (20.6%) was not significantly higher than the total average traumatic injury level (12.3%) for Isla Taboga and Cerro Ancon ($N = 539$, χ^2 contingency test, $\chi^2 = 1.30$, $df = 1$, $0.25 < P < 0.50$).

The two frog species overlapped in size on Isla Taboga (*D. auratus* ranged in snout-vent length (SVL) from 24.4–33.4 mm, mean = 29.1 mm, $N = 163$, whereas *P. pustulosus* range from 27.8–48.2 mm, mean = 31.8 mm, $N = 180$), although *P. pustulosus* was significantly larger (one-way analysis of variance for SVL data from 1999, $F = 132.5$, $df = 1$, $P \ll 0.001$). There were significant interpopulation differences in size within both species. Isla Taboga *P. pustulosus* were significantly larger than the nocturnal *P. pustulosus* from Cerro Ancon (one-way analysis of variance for SVL, $F = 142$, $df = 1$, $P \ll 0.001$). For both years, there were significant differences in size between the three populations of *D. auratus* (one-way analysis of variance for SVL in 1998, $F = 96.3$, $df = 2$, $P \ll 0.001$ and 1999, $F = 59.5$, $df = 2$, $P \ll 0.001$). Overall, *D. auratus* from Cerro Bruja were larger than those from Cerro Ancon, which were larger than those from Isla Taboga. Although there were significant differences in the sizes of frogs between species and between populations of the same species, most of the known predators of *P. pustulosus* and the potential predators of *D. auratus* are much larger than either species of frog so the size differences detected here should not restrict predation (Ryan, 1985; Gray et al., 1999; Summers, 1999).

Of 111 recently metamorphosed juvenile *P. pustulosus* captured on Isla Taboga, three had traumatic injuries (one digit amputation, two foot amputations). Although the percentage of metamorphs with injuries (2.7%) was lower than that found in adults (4.3%), it is not significantly so ($N = 111$, χ^2 contingency test, $\chi^2 = 0.14$, $df = 1$, $0.50 < P < 0.75$). Only 10 *D. auratus* juveniles were captured. Although one juvenile was missing two digits, the sample size is insufficient for statistical treatment.

To take life span into account, the level of injury for each species was divided by the life span of the frog. Based on mark-recapture data of frogs in the wild, *P. pustulosus* usually lives less than one year, whereas *D. auratus* lives about three years (D. Marsh, K. Summers,

pers. comm.). If one crudely divides overall trauma rate by life span, the rate of traumatic injury is 4.4% for *D. auratus* and 4.3% for *P. pustulosus* (Table 1).

Traumatic injury rates in large-scale studies of anurans generally fall below 5% (Rostand, 1949; Dubois, 1979; Read and Tyler, 1994; Ouellet, 2000). In French populations of *Rana esculenta* complex, Dubois (1979) found 4.6% of adults ($N = 1040$) had traumatic injuries, whereas in populations of *Bufo bufo* 1.0% of males ($N = 44000$) captured had digit abnormalities (Rostand, 1949). The rate of trauma for *P. pustulosus* at 4.3% falls within this range. The rate of injury for *D. auratus* adults at 13.3% is high compared to the rate for *P. pustulosus* as well as those rates previously documented.

If predator interactions are the source of injuries, a difference in traumatic injury levels between these two species is not surprising given that the species have very different antipredator strategies. *Dendrobates*, which possess potent skin toxins that would make them unpalatable to predators (Daly et al., 1978), will produce visible amounts of skin secretions when threatened by theraphosid spiders (Gray, 2000). When threatened by spiders, *P. pustulosus* will puff its body up with air making itself much larger and presumably more difficult to handle (Gray, 2000). Before differences in antipredator strategy and predator response are used to explain differences in the levels of injury, the basic assumption that traumatic injury rates accurately reflect predation pressure must be satisfied. When the source and nature of the traumatic injuries was examined, this assumption seems unjustified. When *D. auratus* were attacked and then released by the spider *Sericopelma rubronitens*, the only injuries they ever sustained were scrapes from fangs on the torso that subsequently healed (Gray, 2000). The vast majority of injuries found in both frog species are to digits, suggesting there may be a common source. There are a myriad of predators known for *P. pustulosus* (Ryan, 1985), whereas there are very few described predators of the toxic *D. auratus*, and the one predator examined does not produce injury to digits, suggesting that the common source may not be a shared predator. In addition, it is unlikely that unsuccessful predation would result in such a minor injury as the partial loss of a digit, implying that predation is not the primary cause of injury in the frogs studied.

The most likely sources of the injuries are small invertebrates, such as ants, small spiders, and small crabs, which are neither prey nor predators but share the habitat. *Dendrobates auratus* have been caught in the field with ants firmly clamped to the tips of digits (Gray, 2000). Interactions with ants were often observed while males were transporting tadpoles to waterpools, when frogs that approached ants would be chased away (Dunn, 1941; Gray, 2000). Another possible source of digit trauma, to males transporting tadpoles particularly, may be through interactions with small crabs such as *Armases angustum*. This crab was found inhabiting a treehole and readily consumed *D. auratus* tadpoles and could pose a risk to males entering the water to deposit tadpoles (Gray and Christy, 2000). Small invertebrates, such as leeches and small bivalves, have been found to be the source of digit injury to a number of temperate amphibian

species (Dubois, 1979; Davis and Gilhen, 1982; Veith and Viertel, 1993).

Traumatic injuries accumulate over time; therefore the level of trauma for juveniles is expected to be lower than that for adults. An increase in traumatic injury with age has been shown in French populations of *Rana esculenta* complex where froglets had injury rates of 2.9% and adults had injury rates of 4.6% ($N = 2524$ froglets and 1040 adults; Dubois, 1979). This trend is seen in *P. pustulosus* as 2.7% of metamorphs had injuries which was lower than the level in adults (4.3%).

As the reproductive period of an animal's life is the most active, it is expected that more injuries would occur during this period. For *D. auratus*, the reproductive period is one of male territory defense, female search for mates, lengthy courtship for pairs, male exploration for suitable pools, and transportation of tadpoles to these pools. The reproductive period is one of greater movement for *P. pustulosus* as well. Males must move through the forest to water to call, whereas females move to the water to mate with the calling males. Both then move away from the water. For both species, the adult life stage involves extensive moving about, which would expose the frogs to many potential aggressive interactions with other forest dwellers. A trend for increased risk of injury to reproductive adults is seen for *D. auratus*. The recapture data for *D. auratus* show that the rate of new injury over a full year (20.6%) is higher than the total average traumatic injury rate on Isla Taboga and Cerro Ancon (12.3%).

Although the absolute rates of trauma are different between the two frog species, when the life span of each species is considered, the differences become less obvious. The age-controlled rate of traumatic injury is very similar between the two species (Table 1). Although this rate does not equal the observed yearly accumulation rate for *D. auratus*, as seen from the recapture data, it does imply that more work is needed on the demographics and age-specific accumulation of injury. If the proportion of animals in each life stage and the proportion of life span spent in each stage are approximately the same for the two species, then the rate of injury accumulation is a function of the time spent moving about the environment. Further testing of traumatic injury levels in ecologically similar species of frog which differ in life span is needed.

These frogs provide evidence that traumatic injury is not a result of unsuccessful predation attempts. The nature of their injuries is minor, and the types of injury are the same in the two species of frogs, although they have different predators and antipredator strategies. The toxic, brightly colored *D. auratus* has very few known predators in the wild, whereas *P. pustulosus* is palatable and cryptically colored and has a wide range of known predators. Such differences in predators would likely result in differences in the types of injuries seen if predators were their cause. The primary source of injuries for the two species of frogs is therefore not predation but is most likely the myriad of small invertebrates that interact aggressively with the frogs. When comparing levels of injury, careful consideration of life history must be exercised as traumatic injury is accumulated and therefore is a function of a species' use of time and space.

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A New Reproductive Mode for the Genus *Phrynobatrachus*: *Phrynobatrachus alticola* Has Nonfeeding, Nonhatching Tadpoles

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The genus *Phrynobatrachus* is one of the most diverse African anuran genera. Currently approximately 66 species, occurring exclusively in Sub-Saharan Africa, are recognized (Poynton, 1999). Despite the fact that members of this genus inhabit habitats ranging from dry savannas to primary rain forests and a wide range of altitudes, their reproductive patterns are astonishingly uniform so far as is known (Altig and McDiarmid, 1999). With the exception of *Phrynobatrachus guineensis* that deposits its few large eggs in small water-filled tree holes (Rödel, 1998), all other species, oviposit clutches of several hundred to a few thousand small eggs that float in a single layer on the surface of stagnant or slow running water (Stewart, 1967; Wager, 1986; Rödel, 2000a). All tadpoles described are exotrophic, lentic, and benthic (Altig and McDiarmid, 1999; Rödel, 2000a, unpubl. data).

In 1993, we collected a *Phrynobatrachus alticola* female in Southern Taï National Park (TNP, 5°08'–6°07'N; 6°47'–7°25'W), close to the village of Guiroutou, Ivory Coast. In captivity, it deposited large eggs rich in yolk, that indicated a reproductive mode dif-

ferent from those of other members of the genus. The possibility of a reproductive mode independent of water was already discussed by Schiøtz (1964b) based on his observation that males always vocalized far from open water. In January 1999, we started an amphibian monitoring program in TNP. In the meantime, 13 *Phrynobatrachus* species have been recorded from this park (Rödel, 2000b). *Phrynobatrachus alticola*, originally described in 1961 by Guibé and Lamotte, from Mont Nimba, Guinea and Mont Tonkoui, Ivory Coast, is one of the most common anuran species in TNP. During our field season from March to October 2000, we were able to demonstrate that *P. alticola* tadpoles undergo an alternative development. While searching for adult *P. alticola*, on 5 June 2000, we discovered a gelatinous mass (no. 1) with two juvenile *P. alticola* on a dead leaf. In the following month, we regularly checked the leaf litter for clutches in those areas of the forest where *P. alticola* was common. On 13 September, we discovered two leaves with clutches (no. 2 and 3) that were reared in a terrarium at the field station.

The TNP is the largest protected area of rain forest in West Africa. Yearly precipitation reaches 2200 mm in the southwest and 1700 mm in the northeast of the park. Most precipitation occurs from April to July and from September to November. The first dry period lasts from December to February, normally a second one occurs in August. Temperatures vary between 20 and 33°C, with daily temperature differences of up to 10°C. The mean annual temperature is about 25°C. Humidity fluctuates from 85% (day) to 90–100% (night). This area is situated within the equatorial climate (Riezebos et al., 1994), floristically it belongs to the Guinea-Congo-Region (Guillaumet, 1967). Our main investigation area was located about 23 km southeast of the small town of Taï and comprises about 30 km² of primary and secondary rain forest around the Station de Recherche en Ecologie Tropicale (SRET, 05°50.003'N, 007°20.536'W, formerly CRE and IET). Precipitation at the SRET station was 1820 mm in 1999 and 1494 mm from 1 January to 17 October 2000 (R. Noë, pers. comm.).

Data were collected irregularly in different parts of the forest and regularly along 10 transects, 600 m in length. Six transects were in primary and four in secondary forest. Data collection and transect conception were described in more detail in Rödel (2000b). Larvae of different stages were preserved in 4% formaldehyde and transferred to 70% ethanol after two months. We preserved 19 larvae and juveniles, and by comparing the embryonic development in the clutches 2 and 3, we established an approximate developmental timetable based on Gosner (1960). Measurements were taken with a dial caliper (± 0.1 mm) or a measuring ocular in a dissecting microscope (± 0.1 mm, Zeiss Stemi SV 6). Drawings were done with the aid of a camera lucida. One tadpole (stage 31) was additionally examined by means of a scanning electronic microscope. All specimens were deposited in the collection of the Staatliches Museum für Naturkunde Stuttgart (SMNS; Appendix 1). Species affiliation of the clutches was assured by rearing hatched juveniles. These showed most of the characteristic features of adult *P. alticola* (Fig. 1A–B,D).

Adult description.—*Phrynobatrachus alticola* is a small frog. Males had a mean snout-vent length (SVL) of

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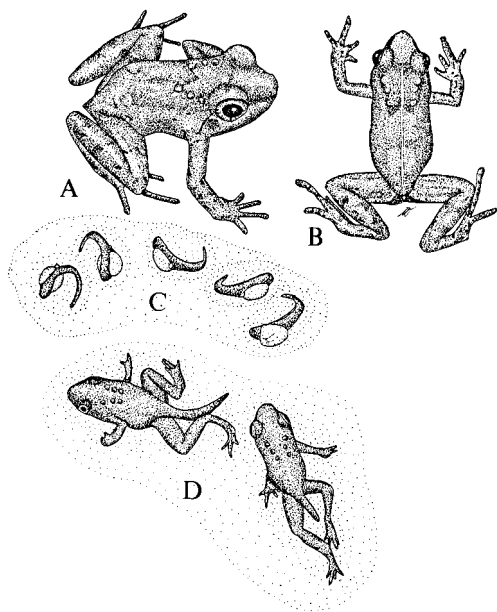


FIG. 1. (A) adult male and (B) female (SMNS 9732) *Phrynobatrachus alticola* from Tai National Park, (C) five larvae in a gelatinous mass about eight days after egg deposition, each larva separated by two individual jelly layers, (D) two juveniles, about 25 days old, shortly before hatching but still wrapped by an individual and a communal layer. Drawn from color slides.

15.6 \pm 1.3 mm (range: 12.5–18 mm, $N = 27$), females were 19.6 \pm 0.9 mm (17.9–20.5, $N = 10$) and significantly larger than males (Mann-Whitney U -test, $Z = -4.605$, $P < 0.001$, $N = 37$). They normally are uniform beige, brown or copper-red dorsally. Breeding males may be nearly black. A few individuals show either a narrow pale vertebral line (Fig. 1B) or a broad, pale dorsal band that are orange to red and extending from between the eyes or the snout tip to the vent. Dark bars on the extremities are most often inconspicuous. *Phrynobatrachus alticola* lacks an eyelid cornicle and foot webbing. Its most diagnostic characters are three pairs of dorsal warts that are not present in a comparable arrangement in any other West African *Phrynobatrachus* (Fig. 1A–B,D) and the golden glitter of its eyes. The warts often become inconspicuous in preserved specimens.

Distribution and Habitat Selection.—*Phrynobatrachus alticola* is endemic to the Upper Guinean rain-forest zone, West Africa (Lamotte, 1966). It has been recorded from Sierra Leone, Liberia, Guinea, Ivory Coast, and one locality in Western Ghana (Guibé and Lamotte, 1961, 1963; Schiøtz, 1964a,b; Lamotte, 1971, pers. obs.). It inhabits rain forests in lowland and medium altitude up to 1600 m above sea level (Guibé and Lamotte, 1963; Lamotte, 1971). During 18 months of fieldwork in 1999 and 2000, we registered 2005 *P. alticola* with a mean frequency of 19 specimens per transect hour in secondary (1810 frogs in 93 h) and one specimen per transect hour in primary habitats (195 frogs in 289 h). Thus its preferred habitats were secondary forests with a broken canopy and dense un-

derstory. In none of its habitats was open water available nearby. On rare occasions, *P. alticola* occurs in dense savanna habitats (Sierra Leone: Schiøtz, 1964b; Lamotte, 1971; Guinea: Guibé and Lamotte, 1963; Ivory Coast: Parc National de Mont Sangbé, 08°01.305'N, 07°23.949'W, pers. obs.).

Life History.—*Phrynobatrachus lticola* was diurnal and males vocalized during almost the entire rainy season. Typical calling sites were established between leaves on small elevations on the forest floor that were always separated by several meters from each other (Schiøtz, 1964b; pers. obs.). Neighboring males vocalized alternately (pers. obs.). The advertisement call has been described by Schiøtz (1964c); he noted some differences in the call structure among populations. We found three clutches of *P. alticola*; all were attached to dead leaves lying on the forest floor. The edges of the leaves were curled up, and the clutches were at least partly hidden. The leaf litter normally was very wet in these areas. In all cases, a single *P. alticola* male called in close vicinity to the clutches. The smallest *P. alticola* that we found in the field measured 5 mm SVL. Froglets of that size were continuously found from early June to late September.

Development.—Clutch 1 contained two froglets (SVL including tail 8.2 mm), and an unfertilized egg (diameter: 3.2 mm; egg diameter always without jelly coats). The two froglets hatched three days later (SVL: 3.5 and 4.5 mm). Clutch 2 comprised seven eggs (stage 16) and clutch 3 comprised seven larvae (stage 34–35). All three clutches consisted of a large watery, gelatinous mass. The eggs, larvae and froglets were embedded within this mass. They were additionally surrounded by two jelly coats each (Figs. 1C–D, 2A). Clutch 2 measured 37 \times 28 mm in total. The diameter of the white to light yellow eggs was about 4 mm ($N = 7$). Embryos of stage 19 (Fig. 2A) measured 3 mm, their yolk sacks measured 1.8 \times 1 mm ($N = 1$). The ground color of the larvae was light gray to gray-brown; the yolk was yellow. After five days, the larvae reached approximately stage 31 (Fig. 2B–D). Their body length (BL) was 2.7 mm; total length (TL) 7.5 mm. Buds of hind limbs (HL) measured 0.6 mm; fin depth (FD) was 1.1 mm ($N = 1$). Eyes were present and nostrils were visible on the ventral side just in front of the open mouth (Fig. 2D). The dorsal fin originated anterior to the tail-body junction. The vent opened medially. Behind the eyes two light spots were distinct (Fig. 2C). Four days later the larvae were at stage 34–35, the stage of the larvae of clutch 3 when discovered (Fig. 2E–G). At stage 34–35 the larvae measured 3.0 \pm 0.2 mm BL, 9.9 \pm 0.5 mm TL, 1.4 \pm 0.1 mm FD and 1.1 \pm 0.1 HL ($N = 5$). The nostrils were then in a more lateral position (Fig. 2E). The mouth was bordered by anterior and posterior lips. Jaw sheaths were present but weakly keratinized and nearly transparent with black edges. Keratodonts and papillae were absent (Fig. 2G). Buds of forelimbs were visible through the ventral skin (Fig. 2F). The dorsal fin now originated in a more posterior position at the tail-body junction. The larvae showed movements but were separated from each other by their individual jelly layers. Five days later stage 41 larvae measured 3 mm BL, 10.5 mm TL, 1.1 FD and 3.4 HL (Fig. 2H–I, $N = 1$). The forelimbs were ready to break through the skin (Fig. 2I). Froglets started to metamorphose

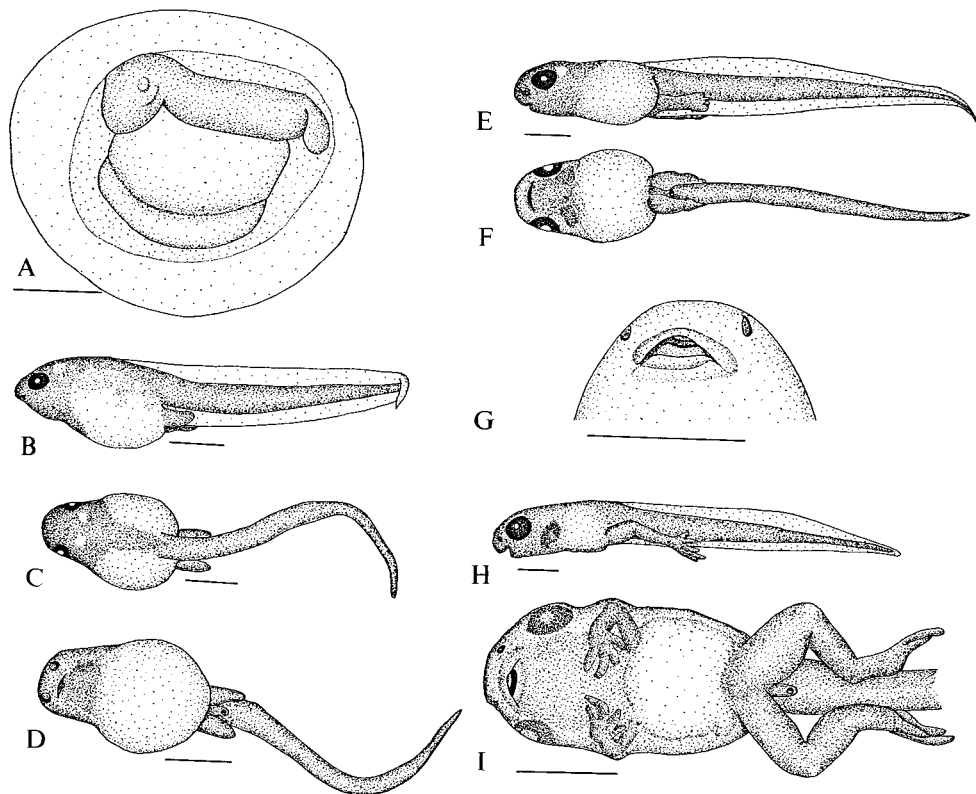


FIG. 2. (A) *Phrynobatrachus alticola* about two days old, five-day postdeposition larvae in (B) lateral, (C) dorsal, and (D) ventral views, 12-day-old larvae in (E) lateral, and (F) ventral views, (G) mouthpart, a 20-day-old larvae in (H) lateral, and (I) ventral views. Drawn from preserved prehatching specimens. Scale bars = 1mm.

and hatch 26–28 days after spawning, most of them had remnants of the tail (Fig. 1D). At that time they measured 3.3 ± 0.1 mm BL, and 6.7 ± 2.7 mm TL ($N = 10$). Their HL were 5.2 ± 0.8 mm long ($N = 8$). The three pairs of dorsal warts were easily visible and the eyes had the golden glimmer of adult frogs.

The available data show that *P. alticola* has endotrophic larvae, a hitherto unknown reproductive mode for the genus. Endotrophic anurans obtain their entire developmental energy from parental sources, most commonly vitellogenic yolk (Thibaudeau and Altig, 1999). *Phrynobatrachus alticola* best fits the direct developer guild 6, as defined by Altig and Johnston (1989): “eggs oviposited terrestrially, embryo develops from oogenic energy sources to produce various sorts of free-living, non-feeding larvae.” The development of *P. alticola* is unique in that the tadpoles did not hatch until they nearly completed metamorphosis. The few other African anurans known to have endotrophic larvae include all *Arthroleptis* species (e.g., Guibé and Lamotte, 1958; Lamotte and Perret, 1963; Barbault and Trefaut Rodriguez, 1979; Wager, 1986), two species of *Arthroleptella*, *Anhydrophryne rattrayi* (Lamotte and Lescur, 1977; Wager, 1986), *Phrynodon sandersoni* (Amiet, 1981), some microhylid frogs and some bufonid toads (for recent review see Thibaudeau and Altig, 1999).

Despite their developmental mode tadpoles of *P. alticola* did not appear different, compared to other,

free-living, exotrophic tadpoles of the genus. The most obvious differences were the incomplete differentiation of the tadpole’s mouthparts and the lack of external gills and a spiracle. The long tail might have served as a respiratory organ, but we did not observe special vascularization of the tail. In other endotrophic larvae, the loss of tadpole characters is often more striking (e.g., buds of forelimbs often develop earlier, *Eleutherodactylus* and *Arthroleptis*; Thibaudeau and Altig, 1999) the larvae have a much smaller mouth that lacks jaw sheaths and keratodonts (e.g., *Phrynodon sandersoni*, Amiet, 1981). *Eleutherodactylus* lacks any vestiges of tadpole mouthparts, whereas *Arthroleptella* and *Nimbaphrynoides* still possess vestiges of such oral structures (Thibaudeau and Altig, 1999).

Its developmental mode makes *P. alticola* comparatively independent from rainfall and open water. This is reflected by its selection of relatively dry habitats, such as secondary forests and habitats in high altitude, as well as by their obviously continuous reproductive activity. Despite the abundance of *P. alticola* in some areas (juveniles were regularly recorded) we only found three clutches, always with a male vocalizing nearby. It is likely that most clutches are much better hidden than those that we found. We are not yet able to say whether *P. alticola* provides some kind of parental care, like *Phrynodon sandersoni*, another petropedetine frog (Amiet, 1981).

The occurrence of different endo- and exotrophic guilds within a recognized genus is unusual (but see Thibaudeau and Altig, 1999). Most other *Phrynobatrachus* have large clutches of small eggs (0.7–1.0 mm), that float on water as a single surface layer (Stewart, 1967; Barbault, 1984; Wager, 1986; Rödel, 2000a). The only known exception is *P. guineensis* that glues its clutches (26.6 ± 12.7 eggs, $N = 25$), of large eggs, rich in yolk (3 mm) above water in small water-filled cavities of trees, empty fruit capsules and snail shells (Rödel, 1998; pers. obs.). We also have data on an undescribed *Phrynobatrachus* from TNP, that deposits its few, large eggs on leaves of living plants or on the forest floor. *Phrynobatrachus alticola*, *P. guineensis*, and *Phrynobatrachus* sp. resemble each other in morphology and composition of their advertisement calls (pers. obs.) and therefore might form a monophyletic group within the West African *Phrynobatrachus*. This group would be characterized by an apomorphic reproductive strategy that makes them semi-independent of aquatic habitats.

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APPENDIX 1

Specimens Examined—*Phrynobatrachus alticola* from Tai National Park, Ivory Coast; specimens from the middle of the park near the town of Tai: SMNS 9729 1–4, 3 males, 1 female, transects IV, V & VIII, 16.III. and 6.IV.2000; SMNS 9730 1–3, males, SRET station, IV.1999; SMNS 9731 1–2, females, transect IX, VI.2000; SMNS 9733 1–19, larvae and juveniles, IX.2000; specimen from the South of the park near the village of Guiroutou: SMNS 9732, adult female, secondary forest, 1993.

Adult and juvenile *P. alticola* from Liberian and Guinean part of Mont Nimba: MNHN 1998 1547–48, Bord de Cavally, gal. forestiere, Mt. Nimba, Guinée, 2.I.84; MNHN 1998.2695–96, entre grassfield et New Camp ruisseau en forêt, Nimba Expedition, Liberia, 27.IX.66; MNHN 1998.2655, same locality, 20.V.1969; MNHN 1998.1524, 1998.1522, 1998.1527, same locality, 18.VIII.1966; MNHN 1998.2676, same locality, 27.X.1966; MNHN 1998.1533, old Bapa Ouest, Nimba Expedition, Liberia, 21.VII.1966; MNHN 1998.2524, grassfield forêt, 450 m Nimba expedition, Liberia, 20.IX.1966; MNHN 1998.2524 & 2522, Grassfield, Mt. Nimba, Liberia, 500 m, près de la forêt?, 7.VII.1966; MNHN 41 specimens without number, New Mine Road, 700m (Seka valley forest), Liberian Nimba, 4.VII.1969; MNHN 1998.2664 and 2669 and 2670, grassfield, Nimba county, Mt. Nimba, Liberia, 1.VI.1969; MNHN 1998.1197, Nimba expedition, Liberia, grassfield, forêt, 29.X.1966; MNHN 1998.2643 and 2645–2647, Mt. Alpha, Nimba Liberien, 1300 m, 4.V.1969; MNHN 1998.2618–2620 and 2624, Nimba (Liberia), 550–750 m, 28.XII.1965.

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Climatic Correlates of Breeding, Simultaneous Polyandry and Potential for Sperm Competition in the Frog *Crinia georgiana*

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When females mate with more than one male during a period of reproductive receptivity, this creates a situation where the sperm of males must compete to fertilize her ova (Parker, 1970). It is now well recognized that sperm competition can have far-reaching evolutionary consequences for male morphology, behavior, and physiology (Birkhead, 1995). Most work in

this field, however, has focused on species with internal fertilization.

Halliday (1998) noted that among anuran amphibians there is considerable potential for sperm competition. Group matings have been reported in at least 10 species from phylogenetically distinct groups. However, to conclusively demonstrate the operation of sperm competition within a group, it is necessary to show that polyandry leads to multiple paternity (Birkhead, 1995). Evidence for this in anurans is rare. D'Orgeix and Turner (1995) provided the first example when they used DNA fingerprinting to show that multiple-male amplexus in the red-eyed tree frog *Agalychnis callidryas* resulted in males gaining an equal share of fertilizations. Laurilla and Seppa (1998) claimed that multiple paternity also occurs in the common frog *Rana temporaria*. They used allozymes to estimate paternity from tadpole kin groups and showed that average relatedness was often significantly lower than expected from matings involving one sire. However, paternity analysis was not coupled with mating observations; thus, it was impossible to determine whether multiple paternity resulted from several males amplexing a female or from sperm diffusing from pairs mating nearby.

Recently, Roberts et al. (1999) provided the second direct example of multiple paternity in the West Australian myobatrachid frog *Crinia georgiana*. Using allozyme electrophoresis, they demonstrated that, during group spawns, males amplexed dorsally and ventrally with a single female acquired an equal share of paternity, but other males did not fertilize eggs. Approximately half of all matings involved multiple males in this species. This estimate was based on observations made in four breeding populations. Three of these populations occurred at the northern end of the species range with only one single small sample from the center. Mating behavior can vary greatly between populations at the extremes of a species' range as well as within populations between breeding years caused by environmental factors influencing the spatio-temporal distribution of males and females (Emlen and Oring, 1977). Therefore, to determine whether polyandry is a common feature of mating behavior, it is necessary to conduct behavioral observations within different populations across the species' range. Identifying the ubiquity of polyandry is an essential first step toward understanding the adaptive significance and evolutionary consequences of this behavior.

To date, factors generating simultaneous polyandry in anurans remain largely unknown. In several species, amplexus by additional males has been interpreted as a strategy used by competitively inferior males to sneak fertilizations (for references, see Halliday, 1998). Within anuran breeding aggregations, the intensity that males compete for mates has been shown to depend on male density and the degree of male bias in the operational sex ratio (OSR; Wells, 1977; Halliday and Tejedo, 1995). Because male chorus attendance and female arrival patterns are usually dependent on climatic conditions, temporal fluctuations in male density and OSR occur between and within episodes of breeding activity (Wells, 1977; Arak, 1988). In *C. georgiana*, field observations have shown that variation in both male density and the OSR within aggregations significantly influences the number of am-

plectant males in a mating (unpubl. data). Given this association, a strong link between climatic conditions and polyandry is expected.

The aim of this investigation was to clarify the potential for sperm competition in *C. georgiana* by documenting temporal patterns of reproductive activity and the incidence of simultaneous polyandry in relation to climatic conditions within a population over the duration of a single breeding season. An additional aim was to determine the frequency of multiple-male amplexus in populations distributed across the species' range and whether frequencies differed within populations between breeding years.

Explanations of temporal variation in breeding activity in anurans often implicate rainfall because of the predominant external mode of fertilization, and the aquatic larval phase generally makes the availability of free water an essential requirement for breeding (Duellman and Trueb, 1986). Because anurans are ectotherms, temperature also may be important. Patterns of anuran breeding have also been linked to lunar phase (Church, 1960a,b). To determine the effects of rainfall, temperature, and lunar cycle on the incidence of breeding, I censused activity on 44 nights between June 12 and September 3 1998, in a natural population located near Kangaroo Gully, approximately 30 km east-southeast of Perth, Western Australia.

The breeding site was a series of shallow temporary pools, which formed in drainage lines and depressions on and around a sloping, moss-covered, granite outcrop. The outcrop was divided by a band of low scrub (approximately 50 m in width) forming two discrete breeding areas. The southern area, at the top of the slope, was bordered on all sides by Jarrah bushland and covered an area of approximately 600 m². The northern area, at the base of the slope, was bordered by a stream and covered an area of approximately 800 m². Choruses moved between these two areas. Observations were made between 1800 and 0600 h. I located mating assemblages by haphazardly searching choruses under infrared light using a Nav 3 Nite Vision Scope. When I encountered mating pairs, they were marked using plastic vials to avoid resampling. I abandoned the search if no matings were detected after approximately 90 min. Because amplexus duration is short (average = 23 min, Roberts et al., 1999), it was not possible to record all matings on nights with high breeding activity. Therefore, counts on those nights are likely underestimates of actual mating activity. I obtained information on daily rainfall from a recording station located approximately 8 km southwest of the study site and information on lunar cycle from the Perth Observatory. Variation in temperature was directly recorded on site using an HDL data logger. To estimate nightly temperature, I averaged seven recordings taken at 2-h intervals between 1800 and 0600 h. The effect of rainfall, temperature, and lunar phase on breeding activity was determined using multiple regression. For analysis, number of matings was the dependant and mean nightly temperature (°C), days since rain (> 3 mm), and days since full moon were independent variables. The relationship between these climatic variables and the incidence of multiple male amplexus was also tested using multiple regression analysis, with the pro-

portion of multiple-male matings as the dependant. I omitted nights on which no matings occurred from the analysis because they could not provide proportional information.

Crinia georgiana is widely distributed in forested and coastal areas of southwestern Australia (Tyler et al., 1994). The frequency of multiple-male mating was measured at eight study sites located at extremes of the species range in both 1998 and 1999. All monitoring was conducted between 1700 and 0500 h on nights of breeding between 1 June and 30 September. Sampling involved randomly searching choruses for mating pairs. Searches were abandoned if no matings were detected after ninety minutes. No searches commenced later than 1930 h. Differences in the proportion of multiple-male matings across and within sites between breeding years was compared using repeated measures ANOVA. Proportional data were arcsine transformed for analysis.

To test for temporal trends in the frequency of multiple-male mating within a population across the course of a breeding season, variation within the Kangaroo-Gully population in 1998 was analyzed using simple regression. Days since the start of breeding was the independent and proportion of multiple-male matings was the dependant variable.

Breeding occurred on 21 of the 44 nights censured, with three peaks of activity (Fig. 1). Multiple regression analysis revealed that activity was significantly influenced by climatic conditions ($F_{3,43} = 4.65$, $P = 0.007$, $r^2 = 0.25$). Lunar phase and rainfall, made significant contributions to the model but temperature did not (lunar phase: -0.37 ± 0.11 , $t = 3.25$, $P = 0.002$; rainfall: -0.40 ± 0.17 , $t = 2.25$, $P = 0.029$; temperature: $\pm 0.0027 \pm 0.07$, $t = 0.37$, $P = 0.97$). On nights when breeding occurred, variation in the proportion of matings involving multiple males was not significantly influenced by any of the climatic variables tested ($F_{3,20} = 0.98$, $P = 0.42$, $r^2 = 0.148$).

Averaged over a total of eight sites approximately half of all matings involved two or more males (Table 1). Frequency did not differ significantly between populations across the species range ($F_{7,15} = 1.00$, $P = 0.49$). Within populations frequency also remained constant between breeding years ($F_{1,15} = 1.57$, $P = 0.24$). Within the Kangaroo-Gully population in 1998, temporal variation in frequency was not predictable ($F_{1,34} = 0.09$, $P = 0.76$).

Breeding activity in *C. georgiana* was influenced by climatic conditions. Main (1965) suggested that breeding occurs on cold clear nights after the passage of low pressure systems. Here, analysis of activity within a population over three months showed that breeding was correlated with rainfall, but that temperature was unimportant. The association with rainfall was expected, because *C. georgiana* breeds in shallow temporary pools that form after rain (Ayre et al., 1984). The observation that breeding activity slows considerably at very low ambient temperature (< 2°C) indicates that there may be a lower thermal limit to activity. Because water temperature remains higher than air temperature (unpubl. data), however, there are few nights over the season when activity is likely to be constrained significantly. Interestingly, lunar phase was an important correlate of activity, with significantly more matings occurring close to

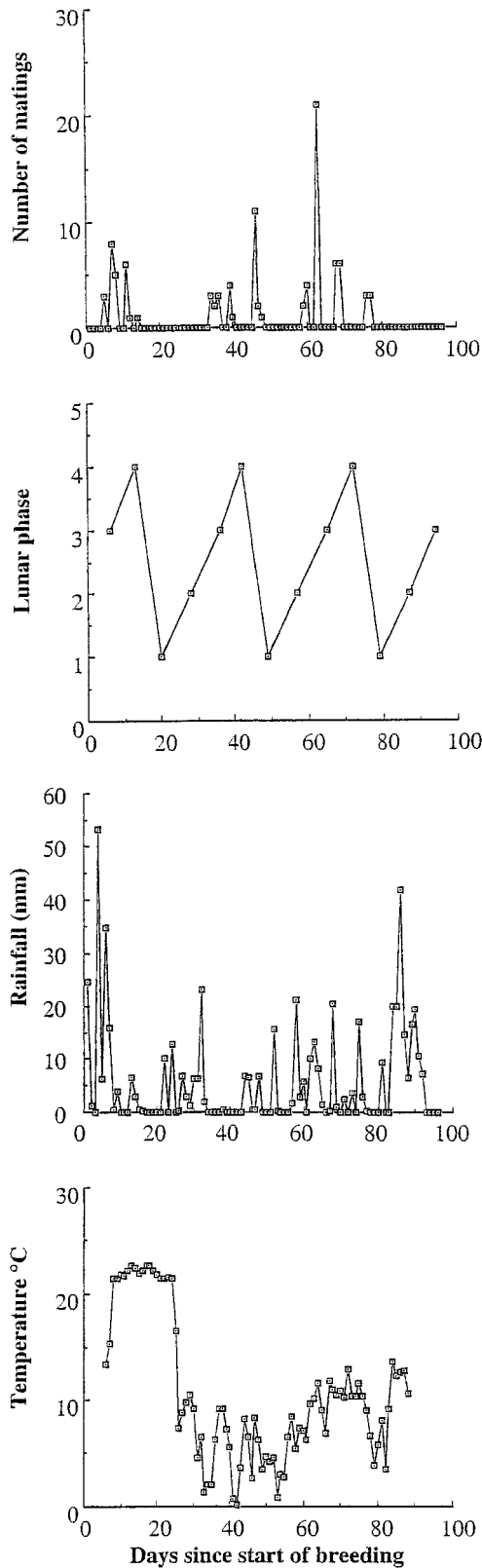


TABLE 1. Frequency of matings involving >2 amplexant males in *Crinia georgiana* at eight study sites over two breeding seasons.

Site	Breeding year	% Multiple-male matings	N
Kangaroo-Gully	1998	56	96
	1999	68	65
Rock Inne	1998	67	6
	1999	40	25
Jarradale (Blue rock)	1998	38	13
	1999	73	11
Ellensbrook	1998	55	22
	1999	47	15
Cowaramup (Tapalinga)	1998	44	9
	1999	38	8
Margaret River east	1998	50	10
	1999	67	12
Margaret River west	1998	60	15
	1999	43	7
Boranup	1998	33	6
	1999	36	11

the full moon. The reason for this remains obscure. It may arise because visual cues are important in female mate choice (Duellman and Trueb, 1986). Alternative explanations may be that ovulation is regulated by the lunar cycle (Church, 1960a,b) or that increased light availability enhances the ability of individuals to detect and avoid predators (Tuttle and Ryan, 1982; Tuttle et al., 1982).

Despite the apparent influence of climatic condition on breeding activity, the incidence of simultaneous polyandry did not vary predictably with any of the factors tested. However, proportions were highly variable, ranging from nights in which all matings observed involved single males to nights in which all matings involved multiple males. Within breeding aggregations changes in both OSR and density have been shown to effect the number of males involved in a mating (unpubl. data). Fluctuations in these variables are likely to be influenced by a number of ecological factors other than climatic conditions. For example, the abundance and distribution of resources critical for breeding (e.g., oviposition sites) may strongly affect chorus dynamics. Investigating the range of factors that influence OSR and male density, and how changes in these variables influence mating behavior, is required to understand the social processes that generate the temporal patterns in polyandry reported here.

Simultaneous polyandry occurs frequently in *C. georgiana*. Averaged over eight study sites, approximately 50% of all observed matings involved two or

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FIG. 1. Nightly mating activity in the frog *Crinia georgiana*, lunar phase, daily rainfall, and average nightly temperature from 5 June to 9 September 1998 in a population near Kangaroo Gully.

more males. This result is in agreement with Roberts et al. (1999), who reported that multiple-male amplexus occurred in an average of 44% of all matings. Although variation in the proportion of polyandrous matings both across populations and within populations between breeding seasons was not significant, considerable variation was evident (under 40% for the Boranup population in 1998 to over 70% for the Jarradale population in 1999). Similar variation also occurred within the Jarradale population between 1998 and 1999. Variation may be explained by the fact that sampling was not conducted on every night of breeding for each population. However, differences in environmental factors that influence social processes are also likely to occur between sites and within sites between years (Emlen and Oring, 1977). Given that the geographic distribution of *C. georgiana* is wide and encompasses a diversity of breeding habitats, ranging from coastal marshes to forest streams (Cogger, 1992), significant variation between sites in ecological factors that influence polyandry are likely. Identifying these factors and how they vary between sites across the entire species range is the next step towards understanding spatial variation in this behavior.

The demonstration that simultaneous polyandry occurs at high frequencies throughout the geographic range of *C. georgiana* is of considerable interest because it indicates that this behavior is a typical feature of the breeding biology of this species. Mating with multiple males may hold a range of benefits to females (Yasui, 1998). However, the likelihood of this being an adaptive female strategy in *C. georgiana* is low. Fertilization success has been shown to decrease as the number of males involved in a mating increases, probably because of competitors interfering with sperm release (Byrne and Roberts, 1999). In addition females are occasional killed by males as they struggle to gain amplexus (Byrne and Roberts, 1999), and laboratory experiments have not revealed any compensatory benefits associated with increasing the genetic diversity of a clutch (Byrne and Roberts, 2000). These findings, coupled with behavioral analysis showing that multiple-male amplexus is driven by intense intrasexual competition (unpubl. data) suggests this behavior occurs against the reproductive interests of females.

Simultaneous polyandry means that males are exposed to a high risk of sperm competition (probability that a female mates with more than one male). Theoretical models postulate that sperm competition should select for adaptive response in sperm allocation strategies. Across species increasing risk of sperm competition is predicted to select for increased sperm expenditure (Parker, 1998). This is based on the assumption that sperm compete in a situation analogous to a lottery and numerical predominance is the solution to out-competing the sperm of rivals. Biological support for this has come largely from the demonstration that, across diverse taxa, testes size increases with risk of sperm competition (Birkhead, 1995). Among anurans, there is some evidence for this association. Kusano et al. (1991) and Jennions and Passmore (1993) showed that rhacophorid species with multiple-male amplexus had larger testes than species in which this be-

havior is absent. However, these investigations did not control for phylogenetic relationships or test for other factors that may effect testes size (Emerson, 1997). Testes mass relative to body mass in *C. georgiana* is at least four times greater than any other *Crinia* species (unpubl. data). Investigating patterns of variation in ejaculate expenditure in relation to risk of sperm competition in *Crinia* and other genera of Australian frogs would provide a valuable contribution to our current understanding of the effects of sperm competition risk on gamete investment in external fertilizers. Because polyandrous matings in *C. georgiana* involve highly variable numbers of males (range = 1–9), the species also provides an excellent opportunity to test recent predictions about how males allocate sperm between matings as the intensity of sperm competition increases (Parker et al., 1996; Ball and Parker, 1997, 1998).

In conclusion, previous demonstration of multiple paternity coupled with the data obtained from this investigation confirms that simultaneous polyandry in *C. georgiana* is a widespread phenomenon and creates a strong potential for sperm competition. Future work should focus on testing how sperm competition influences male gametic investment and allocation strategies.

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Color Pattern Asymmetry as a Correlate of Habitat Disturbance in Spotted Salamanders (*Ambystoma maculatum*)

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Recent work has suggested that amphibian populations are declining worldwide (Lips, 1998; Houlihan et al., 2000). Although global amphibian declines are receiving increased attention, most research focuses on identifying biotic and abiotic causal agents (e.g., Berger et al., 1998; Kiesecker et al., 2001). In addition to identifying agents responsible for declines, we need to identify at-risk populations in a timely fashion such that intervention is possible. One promising monitoring tool is the use of fluctuating asymmetry as an indicator of developmental stability, and ultimately of population stress and fitness (Leary and Allendorf, 1989; Clarke, 1995). If asymmetry proves to be an indicator of population fitness, it could be an inexpensive and useful technique as a precursor to more detailed toxicology and demographic studies of environmental impacts on populations.

Developmental stability is a process by which an organism correctly executes a genetically programmed developmental pathway, producing a predetermined phenotype despite developmental accidents or perturbations (Clarke, 1995). Normal development can be disrupted by genetic and environmental stresses experienced during ontogeny (Palmer and Strobeck, 1986; Leary and Allendorf, 1989), and the ability to buffer against these stresses is an important fitness component (Clarke, 1995). Because both sides of a bilaterally symmetrical trait are expressions of the same genotype, deviations from perfect bilaterality in the terminal phenotype reflect the efficacy of the stability mechanism. Fluctuating asymmetry (FA) is one such measure of stability, defined as the random deviations in right minus left differences in the value of

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a bilaterally symmetrical trait, normally distributed around a mean of zero (Palmer and Strobeck, 1986). Environmental stress during development has been correlated with decreased developmental stability and increased FA in a variety of plants and animals (e.g., Kozlov et al., 1996; Pankakoski et al., 1994; Lens et al., 1999). However, surprisingly little work has focused on applying this tool to amphibian populations.

Many authors have criticized FA as an indicator or signal of individual quality (e.g., Tomkins and Simmons, 1995; Hunt and Simmons, 1997) because quantifiable differences are small and measurement errors are relatively large (Palmer and Strobeck, 1986). However, the case for FA as a biomonitoring tool has been more consistent (Leary and Allendorf, 1989), because total asymmetry tends to be larger and the stress agents more severe (Parsons, 1991). Because of their physiology and complex life histories, amphibians are particularly susceptible to environmental perturbations (Duellman and Trueb, 1994); thus we would expect the correlation between environmental stress and FA to be pronounced in amphibian populations. Two advantages of using FA as a biomonitoring tool are that (1) FA is an additive measure of stress, which is particularly important in the context of amphibian declines where there may be multiple or unknown stress factors; and (2) FA is an early warning system that can be used to detect stress in populations before life-history or demographic parameters are affected. Therefore, simple and relatively inexpensive measurements of FA can be used to determine which populations require management priority (Sarre et al., 1994).

We examined spot asymmetry in two breeding populations of spotted salamanders: one on a golf course and one in a protected wetland. Golf courses have significant impacts on wetlands; studies of golf courses show that aquatic vertebrates accumulate pesticides, herbicides, and fungicides (Tsuda et al., 1988, 1989, 1991) and that these may have detrimental effects on species diversity and population fitness (Goodman et al., 1979; Weis and Weis, 1984). Thus, although golf courses can be managed to reduce the negative impacts on amphibian populations, these habitats are certainly more stressful than undisturbed wetlands in native habitat.

Our goal was to investigate the utility of asymmetry as an assay of disturbance in these two populations. First, we examined the degree of asymmetry in current populations inhabiting the disturbed and undisturbed sites. Second, using museum specimens collected at these same localities before the construction of the golf course, we examined the degree of asymmetry in the past. If color pattern asymmetry is indicative of the degree of population stress, our predictions are threefold: (1) the golf course population will show an increase in asymmetry over the time of the disturbance; (2) the undisturbed population will not show a significant change in asymmetry over the same time period; and (3) these two populations should vary significantly in asymmetry now but not in the past before the disturbance.

We measured spot asymmetry on live adult male spotted salamanders, *Ambystoma maculatum*, from two vernal breeding ponds that are 5.7 km apart and within the same watershed. Individuals from each popu-

lation were collected using dip nets, modified funnel traps, and a drift fence with pitfall traps. We only included adult males in our sample because (1) the sex ratio of males to females in breeding ponds is always male biased, ranging from 1.5–3.5:1 (Petranka, 1998), therefore there were fewer females for comparison; and (2) pooling males and females could introduce bias because of sex differences (for example, gravid females have altered asymmetries caused by skin stretching). Our disturbed site (Bull Pasture Pond, $N = 92$) is on a golf course that was built circa 1954, and the undisturbed site (Ringwood Pond, $N = 97$) is on a nature reserve that has been protected and managed by Cornell Natural Lands since the mid-1920s.

We also measured spot asymmetry on male adult preserved specimens collected from each locality that largely predated the construction of the golf course (1942–1957 for the golf course population, $N = 18$, and 1939–1975 for the reserve population, $N = 12$). Thus, we have two reference populations, one spatial and one temporal, which we use to compare relative asymmetry scores (Clarke, 1995). Because of the geographical proximity of the two populations, it is likely that differences in asymmetry between them are at least partly attributable to the construction and maintenance of the golf course.

Live and preserved animals were photographed in the lab using the macro function on a Nikon digital camera (CoolPix900), and live animals were subsequently released at the site of capture. Measurements were taken from these digital images using NIH Image version 1.52. We measured the area of each spot in square millimeters, and calculated the sum of spot areas on each side of the body from snout to vent. The absolute value of the right minus left difference in total spot area ($|R-L|$) was used as the individual asymmetry score. We measured snout-vent length (SVL) in millimeters for all live and preserved individuals as an index of body size.

Spot area asymmetries for each population, both in the past and present, were normally distributed (Kolmogorov-Smirnov test for normality, $P = 0.3$ for all four populations/time periods). Population variance in spot area asymmetry was significantly larger in the current disturbed population than in the current reserve population (golf course variance 149.36; reserve variance 75.75; F -test: $F = 1.972$, $P = 0.001$). However, variance in spot asymmetry of the two populations collected in the past did not differ significantly (golf course variance 41.24; reserve variance 30.88; F -test: $F = 1.33$, $P = 0.60$). There was a positive correlation between spot area asymmetry and body size (regression of $|R-L|$ area on SVL for populations in the past $F = 16.03$, $df = 1$, $P < 0.001$; for populations in the present $F = 4.58$, $df = 1$, $P < 0.03$). This occurred because larger individuals had larger spots, not more spots (regression of spot number on SVL for past populations $F = 0.45$, $df = 1$, $P = 0.60$; for populations in the present $F = 0.99$, $df = 1$, $P = 0.32$). Adult body sizes (SVL) did not differ significantly between locations in the past or in the present (ANOVA, populations in the past $F = 0.63$, $df = 1$, $P = 0.43$; populations in the present $F = 3.04$, $df = 1$, $P = 0.08$; mean SVL \pm SD: golf course past 82.9 ± 7.1 mm, reserve

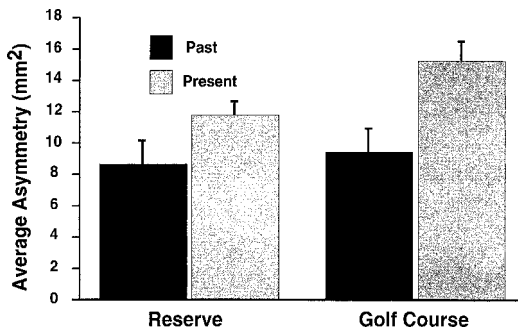


FIG. 1. Mean asymmetry values (R-L spot area asymmetry) for historical and contemporary populations of spotted salamanders (*Ambystoma maculatum*) in disturbed and undisturbed environments. The golf course population is currently more asymmetrical (mean \pm SE = 15.238 ± 1.274) than animals from the undisturbed reference population (11.757 ± 0.884), whereas these two populations did not differ in asymmetry in the past (golf course = 9.403 ± 1.514 , reserve = 8.577 ± 1.604). The golf course population is currently significantly more asymmetrical than it was in the past, whereas the reserve population has not shown a significant change in asymmetry over the same time period.

past 84.9 ± 6.98 mm, golf course present 91.8 ± 7.56 mm, reserve present 89.9 ± 7.39 mm).

Our results confirm the predictions that the golf course population should be more asymmetrical now than before the disturbance occurred, whereas the population on the reserve should not show a significant change in asymmetry (Fig. 1). Adults currently breeding on the golf course are significantly more asymmetrical than individuals collected from that population before the golf course was constructed (unpaired *t*-test, $t = -1.968$, $df = 108$, mean difference between past and present golf course populations = -5.835 , $P = 0.05$). In contrast, adults currently breeding on the reserve are not significantly more asymmetrical than individuals collected from that population in the past (unpaired *t*-test, $t = -1.232$, $df = 107$, mean difference between past and present reserve populations = -3.181 , $P = 0.22$). We also correctly predicted that the disturbed population should currently be more asymmetrical than the reserve population, whereas samples from the past (predisturbance) for the two populations should not be significantly different (unpaired *t*-test, present populations: $t = 2.264$, $df = 187$, mean difference between populations 3.481 , $P = 0.025$; past populations: $t = 0.364$, $df = 28$, mean difference between populations 0.827 , $P = 0.72$, Fig. 1).

Studies using museum specimens collected before and after a disturbance are more informative than those that only compare asymmetry differences in the present (Clarke, 1995; Lens et al., 1999); however, there is some contention over the validity of pooling museum samples through time and space (Swaddle et al., 1994). Our animals were taken from the same two localities; hence, we are only pooling samples over time. Despite our relatively small sample sizes, our results suggest that our historical samples fairly represent

asymmetry in these populations before the construction of the golf course because we did not find significant differences in the past population variances. A second drawback to using museum specimens, especially when measuring coloration characters on amphibians, is that specimens can fade and shrink due to preservation (Klauber, 1943). In fact, it was not possible to score some of the specimens in our collections for these reasons. Shrinkage appears to be a factor in our study because the historical samples were less asymmetrical than contemporary samples, even for the undisturbed population (Fig. 1). Although this result was not significant, it suggests that shrinkage may result in smaller spot areas overall and consequently smaller asymmetries. Nonetheless, the degree of shrinkage should be the same across populations; therefore differences in asymmetry in the past should still be measurable, albeit to a lesser degree. Because of these difficulties associated with the use of museum specimens, we should be cautious with interpreting comparisons across time periods. These measurement errors and artifacts of the preservation process could potentially bias our results because they can be misinterpreted as FA (Palmer and Strobeck, 1986). In this study, we did not duplicate measurements to estimate this error; however, the character we chose shows gross levels of asymmetry (range 0.04 – 54.6 mm²), and, thus, the observed asymmetry differences are not likely biased by measurement error alone.

It is not surprising that the construction of a golf course around a breeding pond can have a negative impact on amphibian populations. The effects of pollutants in vernal pools are often greater than in lakes and streams because vernal pools fill primarily with runoff from surrounding areas and typically have small water volumes and high evaporation rates, leading to increased concentrations of pollutants (Gascon and Planas, 1986; Albers and Prouty, 1987). In addition, the negative effects of pesticides and fertilizers on amphibians have been well documented (Adolfo et al., 1999; McAlpine et al., 1998; Rouse et al., 1999). Our purpose here was to test the utility of asymmetry for detecting these changes in population stress. The spatial and temporal asymmetry differences between these two populations suggest increased stress and decreased fitness in the golf course population. Although a decline has not been demonstrated in the golf course population, our findings suggest that the continued existence of amphibian populations in disturbed habitats does not necessarily guarantee their persistence.

Ecotoxicology and demographic studies of populations are often hampered by the lack of historic baseline data for population or environmental conditions. Historical samples in systematic museum collections are an invaluable resource for measurements of asymmetry and population status for comparison with current populations. Further, these samples allow us to determine the degree to which populations are affected by specific human disturbances. One useful approach would be to survey museum collections for well-represented series from specific localities for comparison with current populations. Amphibians are being touted as sentinels of ecosystem health (Wyman, 1990), and asymmetry has been implicated as an early warning system for monitoring population

health (Clarke, 1995). Combining the two could lead to a powerful strategy for amphibian and ecosystem conservation.

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Descriptions of the Tadpoles of *Scinax alter* and *Scinax cuspidatus* (Anura: Hylidae)

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Duellman and Wiens (1992) recognized *Scinax* Wagler, 1830, as the valid name for *Ololygon* Fitzinger, 1843. They redefined the genus based on the morphology of adults and larvae, osteology, and reproduction. At present, more than 80 species are grouped in this genus, which occurs from Mexico to Argentina (Duellman, 1993). The tadpoles of *Scinax* exhibit a rich diversity of external features. Unfortunately, the larval characteristics of several species in this genus are currently unknown (de Sá et al., 1997).

Scinax alter (Lutz, 1973) and *Scinax cuspidatus* (Lutz, 1925) are similar species that occur in open areas of lowlands and "restingas" (sand-dune vegetation) of Rio de Janeiro State, southeastern Brazil. Herein, their tadpoles are described for the first time.

The specific status of *S. alter* is controversial. Duellman and Wiens (1993) consider this species as a junior synonym of *Scinax ruber* (Laurenti, 1768). However, there are authors who recognize *S. alter* as a valid species, even suggesting that a complex of species could be involved (Carvalho e Silva and Peixoto, 1991; Pomal et al., 1995a,b). None of these authors discussed this problem in detail. We assume that treating *S. alter* as a valid species will cause less confusion at this time, because tadpoles of populations attributed to *S. alter* are different from those attributed to *S. ruber* (Kenny, 1969; Duellman, 1970). This paper is part of a study intended to determine the identity of *S. alter* and *S. ruber*.

We collected adults and tadpoles of *S. alter* and *S. cuspidatus* from Rio de Janeiro State, Brazil. Specimens of *S. alter* were collected from Magé Municipality (22°39'S; 43°02'W), and those of *S. cuspidatus* were collected from Rio de Janeiro Municipality (22°50'S; 43°29'W). We did not find eggs at the collecting sites. Amplexic pairs of the two species were kept in plastic bags for a few hours after they have been collected to obtain eggs. Some tadpoles were reared from these eggs, whereas others were collected in the field. Tadpoles were reared until advanced stages or metamorphosis. Field collected tadpoles were identified by comparisons with those obtained from eggs.

Tadpoles were reared in captivity in plastic boxes

(measurements: 262 × 77 × 147 mm) with about 1.5 liters of water from the collecting sites. Fish food was regularly provided, pH and water temperature were not controlled. Tadpoles were anesthetized in 0.1% chloretone and stored in 5% formalin. Adults were anesthetized in 0.25% chloretone, preserved in 10% formalin, and stored in 70% alcohol. Some eggs were preserved and stored in 5% formalin.

All tadpoles were staged according to Gosner (1960). Tadpoles in stages 36–37 were used in the descriptions and measured. Only tadpoles preserved immediately after capture were used in the descriptions. The measurements, terminology, and labial tooth row formula follow Altig and McDiarmid (1999), except interorbital distance which was taken between the inner margins of the eyes. The term "labial arm" follows McDiarmid and Altig (1990). The technique proposed by Carvalho e Silva and Carvalho e Silva (1994) was used for the study of oral morphology. Eggs were measured without capsules. Drawings were made with the aid of a camera lucida attached to a stereo-microscope.

Specimens examined in this study (listed in Appendix 1) are deposited in the Collection of Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (ZUFRRJ) and at the Natural History Museum, University of Kansas (KU).

Scinax alter (B. Lutz, 1973)

Figure 1

Description.—Mean (\pm SD) total length at stage 36–37, 26.5 \pm 0.8 mm ($N = 23$; Table 1). Body ovoid in dorsal view, approximately as wide as high; maximum height located about three-fourths body length in lateral view. Body length 32% of total length. Snout rounded in dorsal view. External nares small, rounded, located dorsally, and visible in lateral view, slightly closer to eyes than to snout. Internarial distance 82% of interorbital distance. Eyes lateral, their diameter about 31% of body height, interorbital distance 59% of body width and 1.8 times greater than eye diameter. Spiracle sinistral, short, slightly protuberant, located slightly beyond half of body, opening directed posteriorly. Vent tube short, dextral, attached to ventral fin. Tail slightly higher than body, gradually tapering to slender and rounded tip; point of maximum height of tail located about one-third tail length. Tail fins similar in height; dorsal fin arched, gradually ascending from posterior third of body. Oral disc anteroventral, about 36% of body width, with single row of marginal papillae extending ventrally and laterally, and with variable number of lateral submarginal papillae. Labial tooth row formula 2(2)/3(1); posterior labium with third tooth row short, prominent and about 53% of second tooth row ($N = 59$). Jaw sheaths strong and finely serrated.

Color in Life.—General color yellowish-brown covered with numerous dark brown spots and some golden and iridescent spots. Body in lat-

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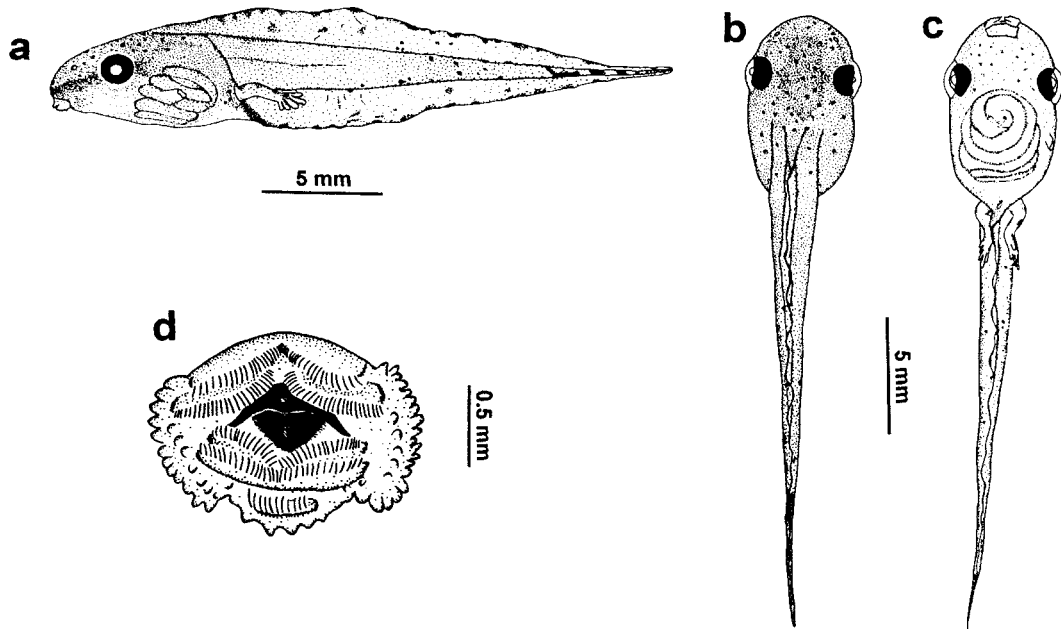


FIG. 1. *Scinax alter* (ZUF RJ 7083). (a) Lateral view, (b) dorsal view, (c) ventral view, (d) oral disc.

eral view with dark brown stripe extending from snout to eyes; eyes coppery-brown. Venter iridescent copper; intestinal coils visible in lateral view. Tail with irregular brown markings. Tail fins translucent yellowish; base of ventral fin with marginal narrow brown stripe.

Color in 5% Formalin.—Body and tail musculature cream covered with dark brown spots and small irregular markings. Dark brown lateral stripe extending from snout to eyes. Spiracle transparent. Venter translucent with intestinal coils visible in lateral and ventral views. Tail with narrow brown stripe at base of ventral fin.

Ecological Notes.—Tadpoles were collected from August to April in semipermanent or temporary ponds in open areas on the edge of lowland forests and in human settlements. In captivity, these tadpoles float throughout the body of water with their bodies horizontal or inclined with the snout pointing upward or downward. They swim with quick movements of the tail tips. Eggs measure about 1.2 mm in diameter ($N = 10$) and are initially bicolored (black in one pole and cream in the other). After a few hours of development, the egg color changes to grayish-brown (embryos present the same color). In

TABLE 1. Measurements (in millimeters) of tadpoles of *Scinax alter* ($N = 23$) and *Scinax cuspidatus* ($N = 13$) in stage 36–37, $\bar{x} \pm SD$ (min–max).

	<i>S. alter</i>	<i>S. cuspidatus</i>
Total length	26.5 \pm 2.1 (22.9–32.0)	32.7 \pm 2.4 (30.0–37.8)
Body length	8.6 \pm 0.6 (7.6–9.7)	10.6 \pm 1.1 (9.6–13.3)
Body height	4.8 \pm 0.3 (4.1–5.6)	5.7 \pm 0.6 (5.0–7.2)
Body width	4.8 \pm 0.3 (4.4–5.6)	5.5 \pm 0.4 (4.9–6.7)
Tail height	5.7 \pm 0.4 (5.0–6.2)	7.1 \pm 0.4 (6.3–7.8)
Dorsal fin height	1.8 \pm 0.2 (1.5–2.1)	1.9 \pm 0.1 (1.7–2.1)
Ventral fin height	1.9 \pm 0.2 (1.5–2.2)	2.6 \pm 0.2 (2.3–2.8)
Eye diameter	1.5 \pm 0.1 (1.4–1.6)	1.7 \pm 0.1 (1.6–1.9)
Nares diameter	0.3 \pm 0.0 (0.2–0.3)	0.3 \pm 0.0 (0.2–0.4)
Interorbital distance	2.8 \pm 0.1 (2.6–3.1)	3.6 \pm 0.5 (3.2–4.6)
Internarial distance	2.3 \pm 0.1 (2.2–2.6)	2.8 \pm 0.2 (2.6–3.1)
Eye–nares distance	1.0 \pm 0.1 (0.8–1.2)	1.4 \pm 0.2 (1.2–1.8)
Eye–snout distance	2.6 \pm 0.3 (1.9–3.2)	3.1 \pm 0.5 (2.6–4.0)
Nares–snout distance	1.5 \pm 0.2 (1.1–1.8)	1.7 \pm 0.3 (1.1–2.3)
Oral disc width	1.7 \pm 0.2 (1.4–2.2)	2.1 \pm 0.2 (2.0–2.6)

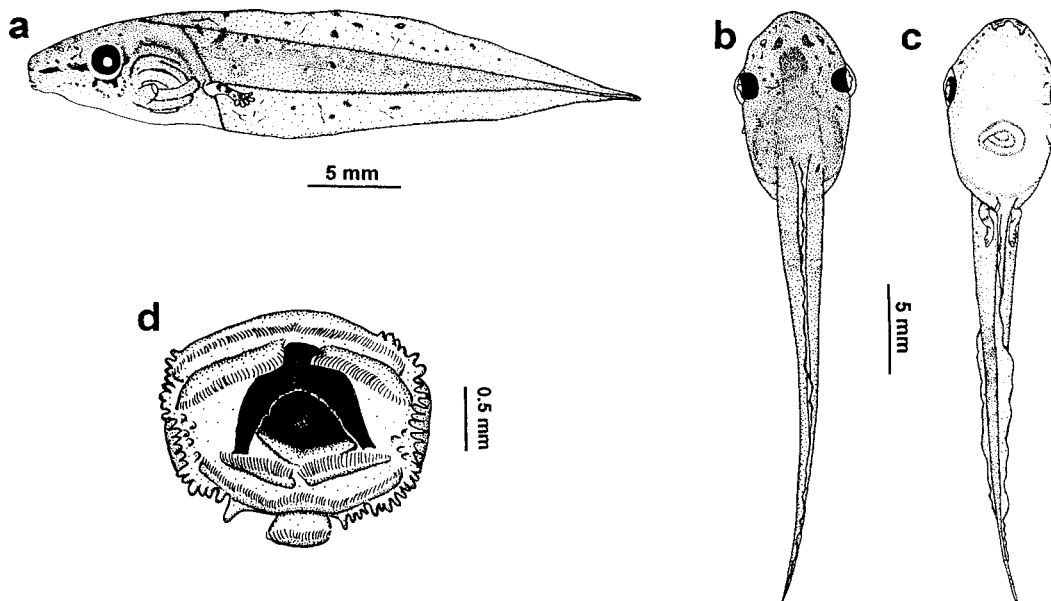


FIG. 2. *Scinax cuspidatus* (ZUF RJ 7450). (a) Lateral view, (b) dorsal view, (c) ventral view, (d) oral disc.

captivity, the embryos are 3.5 times bigger than the eggs after two days, and they reach Gosner's stage 25 five days after the eggs were laid. Mean clutch size was 300 eggs (297–303, $N = 3$); clutches were deposited by females having mean snout-vent lengths of 29.6 mm (28.7–30.7, $N = 3$).

Scinax cuspidatus (A. Lutz, 1925)

Figure 2

Description.—Mean total length at stage 36–37, 32.7 ± 2.4 mm ($N = 13$; Table 1). Body ovoid in dorsal view, approximately triangular in lateral view, with maximum height located about three-fourths body length. Body length 32% of total length. Snout rounded in dorsal view. External nares small, rounded, located dorsally, and visible in lateral view, nearly as close to snout as to eyes. Internarial distance 77% of interorbital distance. Eyes lateral, their diameter about 29% of body height, interorbital distance 65% of body width and about 2 times eye diameter. Spiracle sinistral, short, slightly protuberant, located slightly beyond half of body, opening directed posteriorly. Vent tube short, dextral, and attached to ventral fin. Tail slightly higher than body, gradually tapering to slender and rounded tip; point of maximum height of tail located about one-third tail length. Ventral tail fin slightly deeper than dorsal fin; dorsal fin arched, gradually ascending from posterior third of body. Oral disc anteroventral, about 39% of body width, with single row of marginal papillae interrupted medially at upper and low-

er labia, and with a variable number of lateral submarginal papillae. Labial tooth row formula 2(2)/3(1). Medial part of posterior labium forms a labial arm located in a gap in marginal papillae. Third posterior tooth row at the tip of extended labial arm, its length about 47% of length of second posterior tooth row ($N=25$). Jaw sheaths strong and finely serrated, lower jaw sheath with pair of spiniform, keratinized lateral process located at ends of arch.

Color in Life.—General color greenish-brown to yellowish-brown covered with dark brown and golden spots, usually with slight crescent-shaped mark behind nares. Body in lateral view with incomplete dark brown stripe extending from snout to eyes. Eyes red in lateral view, coppery-brown in dorsal view. Venter iridescent copper; intestinal coils visible in lateral view. Tail with irregular brown markings; tail fins translucent.

Color in 5% Formalin.—Body and tail musculature cream, covered with dark brown spots and small irregular markings. Dark brown lateral stripe extending from snout to eyes; slight crescent-shaped marks behind nares usually preserved. Spiracle transparent. Venter translucent with intestinal coils visible in lateral and ventral views. Tail with irregular brown markings.

Ecological Notes.—Tadpoles were collected from August to April, usually together with the tadpoles of *S. alter*. In captivity, their behavior is similar to that of *S. alter*. Clutch size about 280 eggs (273–286, $N = 2$). Eggs are initially bicolor-

TABLE 2. Measurements (in millimeters) and rate (%) of the third and second posterior tooth rows of the oral disc of the tadpoles of *Scinax alter* ($N = 59$), *Scinax cuspidatus* ($N = 25$), and *Scinax ruber* ($N = 13$). TPR = Third posterior tooth row, SPR = Second posterior tooth row. $\bar{x} \pm SD$ (min-max).

	<i>S. alter</i>	<i>S. cuspidatus</i>	<i>S. ruber</i>
TPR	0.5 \pm 0.1 (0.3–0.8)	0.6 \pm 0.2 (0.3–0.9)	1.2 \pm 0.1 (1.0–1.4)
SPR	1.0 \pm 0.2 (0.7–1.5)	1.2 \pm 0.3 (0.8–1.7)	1.4 \pm 0.1 (1.3–1.5)
TPR/SPR (%)	53.6 \pm 7.6 (37.5–71.0)	47.0 \pm 7.0 (31.6–57.1)	87.1 \pm 4.3 (80.0–93.3)

ored black in one pole and cream in the other, but after a few hours of development, the eggs are yellowish-green (embryos present the same color).

Scinax alter and *S. cuspidatus* usually occur in the same habitats and often are syntopic. The clutches of the two species have a similar appearance, size, and number of eggs but differ from each other in color after a few hours of development. The tadpoles of these species are similar (Figs. 1–2), but tadpoles of *S. alter* are smaller than those of *S. cuspidatus* (see Table 1). Also, tadpoles of *S. alter* can be distinguished from those of *S. cuspidatus* by details of their color patterns as the lateral brown stripe extending from snout to eyes (inconspicuous and incomplete in *S. cuspidatus*) and the marginal, narrow brown stripe at the base of the ventral fin (observed both in live and fixed specimens, absent in *S. cuspidatus*). The fins are about the same size in *S. alter*, whereas in *S. cuspidatus*, the ventral fin is deeper than the dorsal one (Table 1). The oral discs of the two species are remarkably different. The row of marginal papillae is continuous across the posterior labium in *S. alter* (Fig. 1d), whereas in *S. cuspidatus*, it is interrupted medially (Fig. 2d). The third posterior tooth row is about 53% of the length of the second one ($N = 59$) in *S. alter*, and about 47% in *S. cuspidatus* ($N = 25$; Table 2). It is prominent in *S. alter* and located at the end of the extended labial arm in *S. cuspidatus* (Figs. 1d, 2d). A keratinized lateral process at the ends of the arch is observed in *S. cuspidatus* (absent in *S. alter*). This structure was formerly observed in other species of *Scinax* that are now currently included in the *Scinax ruber* group (Alves and Carvalho e Silva, 1999).

McDiarmid and Altig (1990), Hero and Mijares-Urrutia (1995), and de Sá et al. (1997) discussed the labial arm as a synapomorphy of the *Scinax rostratus* group. *Scinax cuspidatus*, traditionally included in the *Scinax x-signatus* group, also has a modified third posterior tooth row supported by a labial arm. De Sá et al. (1997) described the tadpole of *Scinax boesemani*, another member of the *S. x-signatus* group, and reported a labial arm similar to those described for the species of the *S. rostratus* group. The other species included in the *S. x-signatus* group

which have described tadpoles are *Scinax acuminatus*, *Scinax crospeospilus*, *Scinax cruentomma*, and *S. x-signatus* (Duellman, 1972; León, 1975; Heyer et al., 1990). Among these species only *S. crospeospilus* has a labial arm. The oral disc of *S. cuspidatus* is very similar to that of *S. crospeospilus*, but it is quite different from that of *S. boesemani*. Although all three species have a labial arm, the former two ones have the posterior labium with the marginal row of papillae interrupted medially, while the marginal row of papillae of *S. boesemani* has no interruption. Thus, the oral disc of *S. boesemani* is more similar to that of *S. alter*. The prominent posterior labium observed in *S. alter* and *S. boesemani* suggests an intermediate condition between the oral disc of *S. cuspidatus*, *S. crospeospilus*, and the species of the *S. rostratus* group and that observed in the remaining species of the *S. x-signatus* and *S. ruber* groups.

The tadpole of *S. ruber* was described and illustrated by Kenny (1969) and Duellman (1970). Both descriptions are similar, agreeing on the labial tooth row formula (2(2)/3). Nevertheless, the illustration of the oral disc of *S. ruber* presented by Duellman (1970) shows an interrupted first posterior tooth row. Our examination of tadpoles of *S. ruber* (KU109492) studied by Duellman shows that in fact the first tooth row of the posterior labium is interrupted and thus the correct labial tooth row formula is 2(2)/3(1).

The comparison of tadpoles of *S. alter* from Rio de Janeiro State with those of *S. ruber* from Ecuador shows some differences. Tadpoles of *S. alter* have more elongate, but less numerous, marginal papillae. The third tooth row of the posterior labium is prominent and remarkably shorter than the second one in *S. alter* (about 53%) and not prominent and only slightly shorter than the second one in *S. ruber* (87.1%; Table 2). The differences in larval morphology are apparently the first evidence that they are two distinct species. We are now developing a detailed study including data on adult morphology, osteology, and calls.

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APPENDIX 1

Material Examined: *Scinax alter*.—Brazil: **Rio de Janeiro State**, Magé Municipality: ZUF RJ 3101; ZUF RJ 4406; ZUF RJ 6113; ZUF RJ 6265; ZUF RJ 6948; ZUF RJ 7082–7083; ZUF RJ 7112–7113; ZUF RJ 7139; ZUF RJ 7376 (obtained from eggs ZUF RJ 7375, parents ZUF RJ 7245 (♀), ZUF RJ 7248 (♂)); ZUF RJ 7378 (obtained from eggs ZUF RJ 7377, parents ZUF RJ 7373 (♀), ZUF RJ 7374 (♂)); ZUF RJ 7461; ZUF RJ 7495–7496. Maricá Municipality: ZUF RJ 5504. Seropédica Municipality: ZUF RJ 7497.

Scinax cuspidatus.—Brazil: **Rio de Janeiro State**, Magé Municipality: ZUF RJ 7462. Maricá Municipality: ZUF RJ 3307. Ibicuí Municipality: ZUF RJ 1075. Rio de Janeiro Municipality, Vargem Grande: ZUF RJ 7380 (obtained from eggs ZUF RJ 7379, parents ZUF RJ 7255 (♀), ZUF RJ 7258 (♂)); ZUF RJ 7450.

Scinax ruber.—Ecuador: **Napo Province**, NA, Santa Cecilia: KU 109492.

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Seed Dispersal by *Chelonoidis chilensis* in the Chaco Dry Woodland of Argentina

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Many terrestrial and aquatic turtles regularly ingest fleshy fruits (King, 1996). However, only a few recent studies have examined their capacity as seed dispersers (Hnatiuk, 1978; Braun and Brooks, 1987; Cobo and Andreu, 1988; Vuorisalo and Watson, 1990; Milton, 1992; Moll and Jansen, 1995). With the exception of Cobo and Andreu (1988) studies on seed dispersal by tortoises have rarely focused on retention time of seeds within the digestive system of the tortoise, which has important implications on seed viability, germination, distance of dispersal, and spatial pattern of deposition (Barnea et al., 1992; Schupp, 1994; Traveset, 1998).

The common Chaco tortoise, *Chelonoidis chilensis* (= *Chelonoidis petersi* according to Cabrera, 1998) is a fruit consumer in the Chaco dry Woodland of Argentina,

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TABLE 1. Summary of seed passage trials in captive individuals of *Chelonoidis chilensis*. Values represent averages for all trials for each plant species (mean \pm SD).

Parameters	Seed species		
	<i>Z. mistol</i>	<i>P. nigra</i>	<i>C. pallida</i>
Number of seeds ingested	22 \pm 14	18 \pm 4	32 \pm 17
Percentage of seeds recovered	94 \pm 7	88 \pm 11	84 \pm 17
Minimal passage time (days)	6 \pm 3	7 \pm 3	4 \pm 2
Transit time considering all seeds (days)	9 \pm 2	8 \pm 2	6 \pm 2
Number of trials	9	4	5

judged from the frequent occurrence of seeds in its feces (ROV, pers. obs.). Accordingly, it may play a role in the seed dispersal and germination capacity of Chaco plants, but specific studies are lacking. We report here (1) the list of plant species dispersed by the Chaco tortoise in the Chaco forests of northwestern Argentina, (2) the seed passage time through the digestive tract, and (3) the effect of fruit ingestion on germination capacity of woody plant species.

Our study was conducted at the Los Colorados Biological Station (24°38'S, 63°48'W), located in the province of Salta, Argentina. Vegetation at the study site is the typical subtropical, semiarid woodland of the Western Chaco (Bucher, 1982). Annual rainfall is around 550 mm, and mean annual temperature is around 22°C (Bianchi and Yañez, 1992).

We collected feces from 22 wild adult tortoises (14 females and eight males) captured from November to March from 1995 through 1997. Tortoises were housed individually in rectangular cages for 8–12 days, until feces were collected. During captivity, the tortoises were maintained on a varied diet (lettuce, cactus stems, carrot, apples, and oranges, native fruits excluded) and water. Each cage was checked daily for feces. At the end of the experiment, tortoises were released near their capture site. Feces were individually stored in labeled paper bags. Seeds were separated from the feces manually, washed, sun-dried, and observed under a magnifying glass to detect any sign of damage caused during consumption. Seed species were identified by means of a reference collection. To detect seed viability, a random sample of 10 seeds per plant species taken from feces was tested with a colorless solution of 2,3,5 triphenil tetrazolium chloride, used as an indicator of metabolic activity within embryo tissue (Moore, 1972).

Seed passage time through the tortoises' digestive tract was estimated for nine adult individuals (one male, eight females) kept at the Horco Molle Experimental Reserve (HMER), University of Tucumán. Each individual was fed with ripe fruits of the three woody species most commonly found in the feces of wild tortoises (*Ziziphus mistol*, *Celtis pallida*, and *Prosopis nigra*). The fruit of *Z. mistol* is a reddish-brown drupe, with 1–4 seeds covered by an 8 mm woody endocarp. *C. pallida* fruit is an orange drupe with a single seed protected by an almost round, 5-mm diameter, rock-hard endocarp. The fruit of *P. nigra* is an indehiscent yellowish pod, with relatively soft endocarp. One fruit contains 12–25 seeds, each one covered by a coriaceous endocarp of about 8 mm in diameter.

During seed passage trials, each tortoise was kept in a cage for 20 days. Feces were checked for seeds

every 1–3 h each day. Mean minimum retention time was estimated as the average time elapsed from ingestion until deposition of first detected seed of each trial. Mean retention time for each seed species was calculated as $\sum t_i \times f_i / \sum f_i$, where t_i corresponds to seed dropping time for each observation, and f_i is the number of evacuated seeds. Seed passage trials were replicated nine times for *Z. mistol*, five for *C. pallida*, and four for *P. nigra*.

The effect of gut passage on the germination rate of *Z. mistol* seeds and *C. pallida* seeds was evaluated using germination tests under laboratory conditions. We compared the germination rate of seeds collected at random from feces of captive tortoises (ingested seeds) with seeds collected from ripe fruits on the ground (un-ingested seeds). For each treatment (ingested and un-ingested seeds), 10 samples of 20 seeds each were put in individual plastic trays containing moist sterilized sand, in a germination chamber at 32°C. Seeds were watered daily and germination was scored at radicle emergence. The experiment was run for six months.

Feces of Chaco tortoises contained seeds of five different woody species (*Z. mistol*, *P. nigra*, *Prosopis elata*, *Prosopis torquata*, and *C. pallida*). We counted together the seeds of *P. nigra* and *P. elata* because some of them showed intermediate characteristics, making a correct assignment to either species impossible.

Occurrence of seeds in the feces ($N = 22$) was as follows: *Z. mistol*, 73 seeds in 64% of feces; *P. nigra* and *P. elata*, 42 seeds in 50% of feces; *C. pallida*, 31 seeds in 36% of feces, and *P. torquata*, 16 seeds in 11% of feces. None of the seeds showed signs of external damage and 91% of them were viable.

Captive tortoises from HMER swallowed entire fruits without chewing them. In most cases, fruit pericarps in feces showed little physical change compared with their preingestion condition, seeds being still covered by pulp tissue. From 430 seeds ingested by captive tortoises (197 of *Z. mistol*, 72 of *P. nigra* and *P. elata*, and 161 of *C. pallida*), a total of 373 seeds (86.7%) were recovered from feces. Seed passage time for all plant species combined ranged between 3–19 days. Fifty-five percent of the seeds ($N = 237$) was evacuated during the first seven days. Mean minimum passage time (calculated for 19 trials involving nine tortoises) was 5.8 ± 3.1 days, range 3–13 days ($N = 137$ seeds). Mean passage time for all seeds was 8.0 ± 1.9 days, range 3–19 days ($N = 430$ seeds; Table 1). No statistical differences in passage time were found among species (Kruskall-Wallis, $H = 1.79$, $df = 2$, $P > 0.05$).

Total germination percentage of *C. pallida* seeds re-

covered from feces (35.0%) was significantly higher than control seeds (9.6%; Mann-Whitney, $U = 7.0$, $P < 0.02$). There were no significant differences in germination percentages between the experimental (6.4%) and control groups (5%) for *Z. mistol* (Mann-Whitney, $U = 28$, $P > 0.05$).

Our data show that most seeds consumed by the Chaco tortoise remained viable after gut passage, suggesting that this species is a legitimate seed disperser (in the sense of Herrera, 1989). Only five plant species were dispersed by *Chelonoidis chilensis* in this study. This number of plant species is comparable with values reported for some chelonians (e.g., *Terrapene carolina*, six species, Braun and Brooks, 1987; *Rhinoclemmys annulata*, four species, Moll and Jansen, 1995), although lower than the diversity of plant species dispersed by other chelonians (e.g., *Geochelone gigantea*, 28 species, Hnatiuk, 1978; *Testudo graeca*, 34 species, Cobo and Andreu, 1988; *Rhinoclemmys funerea*, 11 species, Moll and Jansen, 1995). Fruit diversity found in feces of *C. chilensis* represented only 15 % of the total number of fleshy fruit species available at the time tortoises were captured ($N = 33$), suggesting that the Chaco tortoise is highly selective in its fruit diet. In our study, seeds of trees and shrubs were the only plant forms dispersed by the Chaco turtle. In contrast, *Testudo graeca* primarily disperses herbs in the Spanish shrubland (Cobo and Andreu, 1988), whereas *Geochelone gigantea* disperses seeds of a wide diversity of living forms, such as trees, shrubs, and herbs in Aldabra (Hnatiuk, 1978).

Seed passage time in *C. chilensis* (average range: 4.6–14.4 days) is longer than the reported values by Cobo and Andreu (1988) for *T. graeca* (average range: 4–8.5 days). Besides the fact that differences in size and shape as well as in the general diet may have influenced passage time in the two tortoises, it is also possible that passage time was underestimated in the case of *T. graeca*. Underestimation may be deduced from the low percentage of seeds recovered (6–34%), which suggests that the observation time was not long enough to allow the 87% recovery rate obtained in our study.

Seed size has been shown to affect seed retention time in the guts of passerine birds (Levey and Grajal, 1991). However, in the present study, no seed size effect was detected, suggesting that seeds of different sizes are dispersed at similar distances.

Our results indicate that fruit consumption by the Chaco tortoise enhances the germination capacity of *Celtis pallida*. Digestion by tortoises might increase the endocarp permeability, but we did not test this directly. It is unlikely that *C. pallida* may have developed a specific adaptation for turtle dispersal, given that its fruits are dispersed by a wide diversity of animal species in the Chaco such as tegu lizards, foxes, seriemas, chachalacas, doves, passerine birds, and others (ROV, unpubl. data). Therefore, the increased germination rate of *C. pallida* could indicate a general response to vertebrate ingestion. On the contrary, the absence of germination enhancement in the case of *Z. mistol* may indicate that either retention time or digestive fluids are insufficient to increase endocarp permeability.

Despite a retention time of over 19 days, the dispersal range of seeds consumed by *C. chilensis* should be limited, considering that the home range of most

terrestrial tortoises is less than 3 ha (Cobo and Andreu 1988; Diemer, 1992).

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Socially Mediated Pitch Alteration by Territorial Male Bullfrogs, *Rana catesbeiana*

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Acoustic communication signals play important roles in aggressive social interactions between male anuran amphibians (Wells, 1977). For example, a number of studies demonstrate that male frogs behaviorally discriminate between two conspecific signals that differ in size-related spectral properties during conflicts over mates, calling sites, or territories (Davies and Halliday, 1978; Arak, 1983; Ramer et al., 1983; Given, 1987). In anurans, spectral call properties, such as dominant or fundamental frequency, are usually negatively correlated with body size because of morphological constraints on the sound producing apparatus (Martin, 1972). Because larger male frogs typically win fights against smaller males (e.g., Howard, 1978; Arak, 1983; Given, 1988), spectral properties of frog calls are often cited as a classic example of an honest, “unbluffable” signal of fighting ability (Wiley, 1983; Alcock, 1998; Bradbury and Vehrencamp, 1998). In playback tests, male frogs are often more likely to persistently attack sources of the high-frequency calls of simulated small males, whereas they typically stop calling or abandon their calling sites in response to the low-frequency calls of a simulated large opponent (e.g., Davies and Halliday, 1978; Arak, 1983; Wagner, 1989).

There is growing evidence, however, that spectral properties of frog calls may be plastic, especially during social interactions among competing males. Lopez et al. (1988) first demonstrated frequency alteration in white-lipped frogs (*Leptodactylus albilabris*), in which males increased or decreased the dominant frequency of their calls to match that of a nearby male. Wagner (1989, 1992) and Bee and Perrill (1996) demonstrated, respectively, that male cricket frogs (*Acris crepitans*) and green frogs (*Rana clamitans*) reliably decreased the dominant frequency of their calls during simulated aggressive interactions over calling sites. Males of the

African painted reed frog (*Hyperolius marmoratus*) lowered the dominant frequency of their calls in a graded fashion in playback tests as the intensity of the playback stimulus is increased (Grafe, 1995). Howard and Young (1998) showed that male American toads (*Bufo americanus*) lower the frequency of their advertisement calls when these are overlapped by the onset of the call of a nearby male. Most recently, Given (1999) demonstrated that male carpenter frogs (*Rana virgatipes*) lowered spectral components of the advertisement calls such that the calls more closely resemble an aggressive call.

Results from these studies suggest that frequency alteration may have some communicative function during aggressive social interactions among male frogs. One hypothesis for the function of frequency alteration is that males lower the frequency of their calls to dishonestly signal their size and fighting ability by attempting to sound like a larger frog (Wagner, 1989, 1992; Bee et al., 2000). If frequency alteration represents a form of dishonest signaling, then we should expect natural selection to favor receivers that devalue spectral cues as honest indicators of male size. Receivers should come to ignore call frequency over evolutionary time. Bee (2002) recently reported that territorial males of the North American bullfrog (*Rana catesbeiana*, Ranidae), unlike many other frogs, do not assess the absolute and relative size of opponents based solely on size-related variation in acoustic signals. Bee (2002) also speculated that male bullfrogs devalued fundamental frequency as an informative size assessment cue because males of this species might also lower the frequency of their calls during aggressive conflicts similar to two other congeners (Bee and Perrill, 1996; Given, 1999). Our goal in this study was to determine whether territorial male bullfrogs alter the fundamental frequency of their advertisement calls during natural aggressive interactions.

During their breeding season, male bullfrogs defend territories that females use as oviposition sites (Howard, 1978). Territory defense consists of advertisement calling, stereotyped movements, physical fighting, and the production of a distinct aggressive vocalization (Emlen, 1968; Wiewandt, 1969; Howard, 1978; Ryan, 1980), which we term an encounter call (Fig. 1; “hiccup vocalization” of Emlen, 1968; “bonk call of Wiewandt, 1969). During agonistic interactions, males direct advertisement calls and encounter calls toward conspecifics that trespass or intrude on their territory. Both call types are produced prior to and between bouts of physical fighting (Emlen, 1968; Wiewandt, 1969; Ryan, 1980; pers. obs.). Males also respond with advertisement and encounter calls to broadcasts of natural and synthetic conspecific advertisement calls (Wiewandt, 1969; Davis, 1987; Bee and Gerhardt, 2001a).

Between May and August 1998, we recorded a total of 1078 advertisement calls (Fig. 1) from 27 male bullfrogs that held territories in ponds located in the Little Dixie Lake Wildlife Area in Calloway County, Missouri. We obtained one set of 20 consecutive advertisement calls on each of two separate recording sessions for each male (median interrecording interval = 2 days, range = 1–16 days). For one male in each recording session, we obtained only 19 calls. Recordings were made with a Sennheiser MKH 70 shotgun mi-

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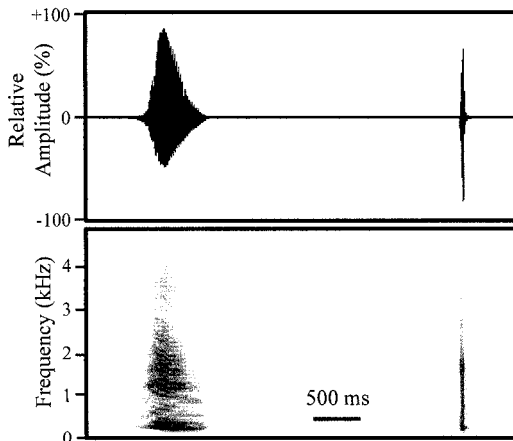


FIG. 1. Oscillograms (upper trace) and sonograms (lower trace) of a one-note advertisement call (left) and an encounter call (right). Horizontal bar indicates time scale.

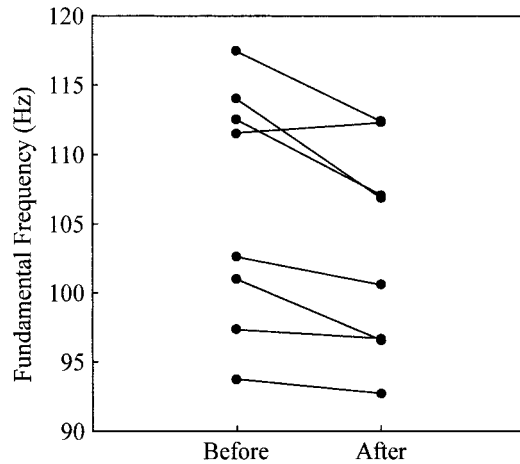


FIG. 2. Fundamental frequency of the advertisement call immediately before and after an encounter call ($N = 8$ males).

crophone equipped with a Windtech SG-3 windscreen and mounted on a tripod placed 1–2 m from a subject. One or two observers sat quietly under ambient light conditions on the bank of the pond approximately 3–6 m away from the subject and recorded calls onto an HHb PDR-1000 DAT recorder (sampling rate = 32 kHz). Prior to recording, all subjects were captured and marked, and their snout–vent length (SVL) and mass were determined.

During the 54 recording sessions (two sessions for each of 27 males), eight males produced at least one encounter call. Because our observations were made at night, and we were positioned several meters away, we could not identify the particular stimuli that elicited encounter calling without disturbing the target male. In previous studies, however, encounter calls were exclusively associated with territory defense and aggressive male–male interactions (Wiewandt, 1969; Ryan, 1980; Davis, 1987; Bee and Gerhardt, 2001a). Therefore, we are confident that the encounter calls recorded in this study were also produced in the context of male–male aggression and territory defense.

To determine whether males changed the spectral properties of their advertisement calls in the context of aggressive signaling, we compared the fundamental frequencies of the advertisement call produced just before and after an encounter call during the recorded sequence of natural calling. If male bullfrogs altered the fundamental frequency of their calls in the context of male–male aggression, we predicted that the fundamental frequency of the advertisement call following the encounter call would be significantly lower than that of the preceding advertisement call. Fundamental frequency ($= 1/\text{waveform periodicity}$) was determined by taking the reciprocal of the average period of five repetitions of the fine-temporal waveform measured from an oscillogram with an expanded time base (± 0.1 ms) using a Kay DSP Sona-Graph Model 5500. If a male produced more than one encounter call during a recording session, we separately determined an average value of fundamental frequen-

cy for the advertisement calls preceding and following the encounter calls.

There were significant negative correlations between SVL and the fundamental frequency of the advertisement calls that occurred both before (Spearman $r = -0.86$, $P < 0.01$, $N = 8$) and after ($r = -0.84$, $P < 0.01$, $N = 8$) the encounter call, indicating that larger male bullfrogs had lower-frequency advertisement calls. There were no significant correlations between fundamental frequency and an index of physical condition calculated as the residuals from a regression of the cube-root of mass on SVL divided by SVL (Baker, 1992; preceding call: $r = 0.07$, $P = 0.87$; following call: $r = 0.31$, $P = 0.36$). There were also no significant correlations between the magnitude of frequency change (in Hz) and either SVL ($r = 0.35$, $P = 0.40$) or physical condition ($r = 0.48$, $P = 0.23$). Similar results were obtained when the magnitude of frequency alteration was measured as a percentage of the fundamental frequency of the preceding advertisement call.

Seven of eight males (87.5%) produced advertisement calls with relatively lower frequencies immediately following the production of an encounter call. In comparison to advertisement calls that immediately preceded an encounter call, advertisement calls that followed an encounter call had significantly lower fundamental frequencies (Fig. 2; Wilcoxon matched-pairs signed ranks test: $T^+ = 34$, $P = 0.012$, $N = 8$). The median fundamental frequency of advertisement calls prior to the encounter call was 107 Hz (range, 94–117 Hz). In the calls following the encounter call, the median fundamental frequency was 104 Hz (range, 93–112). The median percent change in fundamental frequency, measured as a percentage of the fundamental frequency of the first advertisement call, was -3.1% (range, -6.2 – 0.7%). In comparison, the average within-male coefficient of variation ($= \text{SD}/\text{mean} \cdot 100\%$) for fundamental frequency based on the advertisement calls from the first recording session of all subjects was 1.5% (range, 0.6 – 3.9% ; 539 calls from 27 males; Bee and Gerhardt, 2001b).

Our results suggest that in bullfrogs, aggressive

signaling is associated with a decrease in the fundamental frequency of advertisement calls. Although small, the typical magnitude of frequency alteration reported here is clearly audible to both musically trained and naive human listeners (unpubl. data). Whether the bullfrog auditory system can also discriminate between two calls that differ in fundamental frequency by as little as 3–6%, and whether changes in frequency of this magnitude actually play a role in male-male communication are questions that could be addressed in future field playback studies. Previous work has shown that bullfrogs can discriminate differences in fundamental frequency as small as 5–10% (Bee and Gerhardt, 2001a). Wagner (1992) found that male cricket frogs discriminated between two stimuli differing by less than 6% in fundamental frequency.

A number of hypotheses have been proposed for the role of frequency alteration during aggressive interactions in frogs (Wagner, 1992; Bee et al., 2000). According to a call matching hypothesis, male frogs alter the frequency of their own signals to match that of an opponent's calls, as in white-lipped frogs (Lopez et al., 1988). Frequency alteration could represent a form of graded aggressive signaling, as it appears to in painted reed frogs (Grafe, 1995) and perhaps carpenter frogs (Given, 1999). The honest signal of size hypothesis proposes that changes in frequency function as an accurate indicator of male body size (and hence fighting ability) in one of two ways (Wagner, 1992; Bee et al., 2000). First, frequency alteration would honestly signal body size if the lowest frequency a male can produce is a better predictor of body size than the frequency of unaltered calls. Alternatively, males could honestly signal body size if the magnitude of frequency alteration is predictive of body size. Wagner (1992) and Bee et al. (2000) excluded this hypothesis as an explanation for frequency decreases by male cricket frogs and green frogs, respectively. The signal of size-independent fighting ability hypothesis states that the magnitude of frequency alteration indicates a male's true fighting ability, as determined by experience, motivation, or physiological condition, and not by size alone. Based on results from a field playback experiment, Wagner (1992) suggested that male cricket frogs lowered the frequency of their calls to signal their size-independent fighting ability. Bee et al. (2000) suggested this was an unlikely explanation for green frogs. According to the dishonest signal of size hypothesis, male frogs decrease the frequency of their advertisement calls to take advantage of the fact that size is negatively related to fundamental frequency and positively related to the likelihood of winning an escalated contest. Because some male frogs assess the fighting ability of their rivals based on size-related variation in fundamental frequency (Davies and Halliday, 1978; Arak, 1983; Ramer et al., 1983; Given, 1987), lowering fundamental frequency during an aggressive encounter could represent a means of bluffing size. Bee et al. (2000) failed to rule out bluffing as an explanation for frequency alteration in green frogs.

Although the data presented here are too preliminary to rigorously test these hypotheses, several results suggest that some hypotheses are unlikely to explain the function of frequency alteration by male bullfrogs. First, most males lowered the frequency of

their advertisement calls, a result consistent with the observation that males of the closely related green frog consistently lower the frequency of their calls in response to high and low-frequency conspecific calls (Bee et al., 1999, 2000). If in future playback experiments, male bullfrogs were shown to consistently decrease the frequency of their calls, even in response to higher-frequency stimuli, then frequency matching could be excluded as an explanation for frequency alteration in this species. Second, there was no indication of any relationship between the magnitude of frequency alteration and male SVL and physical condition, a result inconsistent with some predictions of the honest signaling hypotheses mentioned above.

Interestingly, fundamental frequency was significantly correlated with SVL in both preceding and following advertisement calls. This result appears at first to be consistent with predictions from an honest signal hypothesis and inconsistent with predictions from a bluffing hypothesis. However, if frequency alteration functions as an honest indicator of size, we should expect the correlations between size and fundamental frequency to improve during periods of aggressive signaling. But this was not the case in this study or in earlier studies of cricket frogs and green frogs (Wagner, 1992; Bee et al., 2000). More important, the fact that fundamental frequency remains correlated with body size in altered calls might be irrelevant if receivers do not also alter the decoding rules they use to estimate an opponent's size and fighting ability based on spectral cues during aggressive interactions. That is, lowered frequencies that are correlated with body size could potentially function as a bluff of size if receivers do not also alter their interpretation of pitch in aggressive contexts. Tests of the effects of frequency alteration on intended receivers during signaling interactions are needed (see Wagner, 1992).

The data presented here lend some support to earlier speculations (Bee, 2002) that bullfrogs ignore size-related variation in fundamental frequency during aggressive conflicts because males lower the fundamental frequency of their advertisement calls during aggressive conflicts. Given the increasing number of studies that demonstrate socially mediated frequency alteration in frogs, and the widespread expectation that fundamental frequency is a static call property that functions as the ideal size assessment cue, further investigation into the plasticity of spectral call properties during aggressive signaling is clearly warranted. The ability to alter spectral call properties may have important influences on the evolution of territorial behavior and communication systems in frogs. Bullfrogs are an attractive anuran species for testing hypotheses about the role of frequency alteration in male-male communication because bullfrogs have an aggressive vocalization that is distinctly different from the advertisement call. This aspect of the bullfrog vocal repertoire thus provides a potential means of examining frequency alteration independently of other measures of male aggressiveness. In addition, bullfrogs are now the third species in the genus *Rana* for which alteration of spectral call properties during agonistic interactions has been documented (Bee and Perrill, 1996; Given, 1999). Future studies should take a comparative approach to examine similarities and

differences in the function of frequency alteration in these close relatives.

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