ECOLOGY AND EVOLUTION OF PHYTOTELM-BREEDING ANURANS

Richard M. Lehtinen
Editor

RECENT *MISCELLANEOUS PUBLICATIONS*

Bailey, R.M., W.C. Latta, & G.R. Smith. 2004. An atlas of Michigan fishes with keys and illustrations for their identification. *Misc. Publ. Mus. Zool., Univ. Michigan*, No. 192, pp. i-iv, 1-215, 219 figs., 4 tables.

Storer, R.W. 2002. The metazoan parasite fauna of loons (Aves: Gaviiformes), its relationship to the birds' evolutionary history and biology, and a comparison with the parasite fauna of grebes. *Misc. Publ. Mus. Zool., Univ. Michigan*, No. 191, pp. i-iv, 1-44, 3 figs., 7 tables.

Albert, J.S. 2001. Species diversity and phylogenetic systematics of American knifefishes (Gymnotiformes, Teleostei). *Misc. Publ. Mus. Zool., Univ. Michigan*, No. 190, pp. i-vi, 1-127, 50 figs.

Nussbaum, R.A. & C.J. Raxworthy. 2000. Systematic revision of the genus *Paroedura* Günther (Reptilia: Squamata: Gekkonidae), with the description of five new species. *Misc. Publ. Mus. Zool., Univ. Michigan*, No. 189, pp. i-iv, 1-26, 12 figs., 7 tables.

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RECENT *OCCASIONAL PAPERS*

Garrison, R.W., N. von Ellenrieder & M.F. O'Brien. 2003. An annotated list of the name-bearing types of species-group names in Odonata preserved in the University of Michigan Museum of Zoology. *Occ. Pap. Mus. Zool., Univ. Michigan*, No. 736, 73 pp.

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Ng, H.H. 2003. *Arius verrucosus*, a new species of freshwater ariid catfish (Teleostei: Ariidae) from the Mekong River. *Occ. Pap. Mus. Zool., Univ. Michigan*, No. 734, 14 pp., 6 figs., 1 table.

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Ecology and Evolution of Phytotelm-Breeding Anurans

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PHYTOTELM-BREEDING ANURANS: PAST, PRESENT AND FUTURE RESEARCH

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INTRODUCTION

As they hatch, they wriggle violently on the moist and polished surface of the banana stem and go sliding down it until they reach the water retained between stem and leaf. Doubtless their progress is often assisted by the frequent showers of rain. They undergo their metamorphoses in the seclusion of this private swimming pool, nourished by the remains of many small insects that are drowned in the fluid.

Barbour & Loveridge (1928, p. 257)

This description of the life history of the east African microhylid *Hoplophryne uluguruensis* was one of the first detailed accounts of a phytotelm-breeding frog. Long thought to be a mere oddity or aberration, egg deposition and tadpole development in water-holding plants is now known to be a somewhat common reproductive mode (found in at least 102 species; Table 1). A phytotelm (from Greek - *phytos* = plant; *telm* = pond; plural phytotelmata) is defined as a water body contained within some part of a plant. Examples include water-filled tree holes, bamboo stumps, bromeliad tanks, nut husks, leaf axils, tree buttresses and other such habitats. These micro-aquatic environments range from being relatively sizeable (tens to hundreds of liters in volume; Schiesari *et al.*, 2003) to extremely small (a few milliliters; Rödel *et al.*, this volume). Phytotelmata are thought to be relatively safe habitats for frog embryos and larvae, compared to ponds or streams where competitors and predators are often abundant. However, predators and competitors can also be found in phytotelmata and these habitats offer many additional challenges such as low dissolved oxygen concentrations, high risk of desiccation and unpredictable food availability.

Anurans that are obligate breeders in phytotelmata have evolved a wide variety of strategies to deal with these constraints. A few of these modifications include: obligatory oophagy (*e.g.*, *Hoplophryne*, Noble, 1929), terrestrial locomotion (*e.g.*, some *Mantidactylus*, Lehtinen, 2004), carnivory (*e.g.*, some *Dendrobates*, Caldwell, 1993), and an array of derived parental care behaviors (Lehtinen and Nussbaum, 2003). These modifications are particularly conspicuous in the larval stage and, in 1987, two of us (MJL and RJW) co-authored with Daniel Townsend a review of what was then known of the tadpoles of these frogs. In the intervening years, much new data have been published and on July 5th 2002, a symposium entitled "Ecology and Evolution of Phytotelmic Anurans" was held at the 50th annual meeting of the Herpetologist's League in Kansas City, Missouri. This symposium gathered twelve speakers to discuss their research on phytotelm-breeding frogs, and is represented here by the papers of Caldwell and de Araújo, Heying, Lehtinen *et al.*, Rödel *et al.* and Summers and McKeon. As a prelude to these papers, we herein review past research, discuss current work and suggest future areas for research on these fascinating frogs.

PAST RESEARCH

A number of early papers briefly mention phytotelm-breeding anurans, usually in the context of other topics. Noble (1927, 1929) was among the first to provide a detailed description and discussion of phytotelm-breeding frogs. Other important papers appeared periodically such as Dunn (1937), Taylor (1954), Scheel (1970) and Wassersug *et al.* (1981). Lannoo *et al.* (1987) published the first summary of this information. In their paper, the authors sought to do two things: 1) explain the unusual morphology of the tadpole of the Jamaican bromeliad-dwelling hylid, *Osteopilus brunneus*, and 2) summarize what was then known about the world's free-living arboreal anuran larvae. This summary resulted in a classification scheme for arboreal tadpoles. Below we review the Lannoo *et al.* paper in order to highlight some of the common themes addressed by the past research of other authors.

Osteopilus brunneus tadpoles live in the tanks of large bromeliads. The morphology of the *O. brunneus* tadpole includes an elongate tail but reduced dorsal and ventral tail fins, a large stomach but reduced intestinal mass, a reduced branchial apparatus including gill filters and gill filaments, enlarged muscles for depressing the jaws and buccal floor but reduced denticles and denticle rows, a v-shaped lower beak, and a reduced lateral line system.

The first goal of Lannoo *et al.* (1987) was to explain the unusual morphology of the *O. brunneus* tadpole, and so they first asked: why the long tail? The tail to body ratio in *O. brunneus* tadpoles is 3.9 and we see similar body elongation in other phytotelm- dwelling larvae. Unlike most tadpoles, *O. brunneus* tadpoles do not grow isometrically, instead tail-length exhibits a positive allometry between Gosner (1960) stages 25 and 31. Three functions have been proposed for the

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long tail.

1) Dunn (1926) proposed a cutaneous respiration function, which Lannoo *et al.* (1987) rejected. The muscular portion of the tail is elongated, but the dorsal and ventral tail fins, which might help to provide respiratory surface area, are reduced.

2) Laessle (1961) proposed that agitation of the elongated tail aerates the water, which Lannoo *et al.* (1987) also rejected, since these tadpoles are typically quiescent.

3) Noble (1929) and Jones (1967) hypothesized that the elongated tail aided locomotion through a viscous medium. Lannoo *et al.* (1987) felt this argument has merit. These bromeliad tanks contain the remains of gelatinous egg capsules and this gelatin-water mixture is viscous. The idea here is that in bromeliad tanks, *O. brunneus* tadpoles may not so much be swimming as they are burrowing. As Gans (1975) has pointed out, elongation is a common evolutionary response to burrowing.

The tail may also function as a postural organ. In the laboratory, Lannoo *et al.* (1987) noted that quiescent *O. brunneus* tadpoles orient vertically. This posture has also been seen in *Acanthixalis sonjae* by Rödel *et al.* (2003) and in bromeliad-dwelling microhylids by Krügel & Richter (1995) and may function to position the head nearer to sources of food and oxygen.

The second question Lannoo *et al.* (1987) asked about the unusual morphology of the *O. brunneus* tadpole regarded the feeding apparatus. In particular, why do *O. brunneus* tadpoles have a shearing beak, reduced denticles and denticle rows, reduced intestinal mass, and an enlarged stomach? Many of these unusual characteristics are also found in other phytotelm dwelling tadpoles. Lannoo *et al.* (1987) found a range of 3–31 tadpoles in occupied bromeliad tanks, in a volume of perhaps half a liter. At densities this high, the question of what to eat becomes important. These authors found that the stomachs of Gosner (1960) stage 25 to 41 tadpoles contained predominantly whole *O. brunneus* eggs, with the number of eggs in 10 stomachs ranging from 5 to 184. Since these eggs showed few signs of developing, Lannoo *et al.* (1987) proposed that these were unfertilized trophic eggs, laid by females as food for their tadpoles. [Thompson (1996) has since shown that eggs laid later in the reproductive season are true trophic eggs, but eggs laid earlier are fertilized and will develop if not ingested.] Lannoo *et al.* (1987) asserted that collectively this morphology reflects a dietary shift from omnivory/detritivory to oophagy. Other phytotelm breeding species have also been shown to have oophagous tadpoles with similar characteristics (Table 1).

The third question Lannoo *et al.* (1987) asked concerned the reduced gill filters and filaments and lack of buccal pumping in *O. brunneus* tadpoles. Noble (1927) suggested that in an aquatic environment that is severely hypoxic (such as bromeliads; Laessle, 1961), increased respiratory surface area is required to obtain sufficient oxygen. However, Lannoo *et al.* (1987) observed that at low dissolved oxygen levels, *O. brunneus*

tadpoles surface to gulp air. Lannoo *et al.* (1987) proposed that reduced surface area in gill elements and tail fins reduces the amount of oxygen *lost* to the water in the bromeliad tank. A tadpole that gulps air and lives in an aquatic environment that is severely hypoxic likely has more oxygen in its blood than there is in the surrounding water, and therefore diffusion gradients favor the loss of oxygen to the environment. In order for *O. brunneus* tadpoles to retain oxygen acquired through air gulping, aquatic gas exchange surfaces are reduced. Given the small size of most phytotelmata, many phytotelm-dwelling larvae probably routinely encounter similar hypoxic conditions. These environmental conditions may account for many of the unusual characteristics of phytotelm-dwelling tadpoles.

The second goal of Lannoo *et al.* (1987) was to modify Orton's (1953) description of arboreal tadpoles as "thin, flattened, larvae" to encompass the variety of arboreal tadpole morphologies described since her early paper. Towards this end, Lannoo *et al.* constructed a table describing denticle pattern, body form, tail/body ratio, diet and habitat–for the roughly 42 species with arboreal tadpoles that were known at the time. From this table, they described a variety of arboreal tadpole types, as follows:

Group I - Elongate tadpoles with attenuate bodies, tail/body ratios >2, denticle formula > 2/3 usually reduced to 1/1 or less; highly reduced gill filters and gill filaments, musculoskeletal specializations for macrophagy or oophagy, little or no pigment. *Osteopilus brunneus* tadpoles and the other Jamaican hylids fit into this group. Arboreal *Hoplophryne*, *Philautus*, and some *Dendrobates* larvae also fit here. Group I tadpoles are primarily associated with leaf axils rather than tree holes. They occur in relatively small volumes of water that have little or no primary productivity as food. All appear to be obligately oophagous, relying on trophic eggs, which are usually unfertilized, but may be fertilized in some species at some times.

Group II - Shorter tadpoles with stout bodies; tail/body ratios > 2; denticle formula reduced to 2/2 or less; highly reduced gill filters and gill filaments; little or no pigment. Among the tadpoles that fit this group are *Hyla zetecki*, *Hyla picadoi*, *Anotheca spinosa*, and the aggressive omnivorous larvae of *Dendrobates quinquevittaus*. These tadpoles are carnivorous and macrophagous and are associated with bromeliads that have little free water. Conspecific frog eggs may form a major portion of their diet, but the evidence for obligate oophagy and trophic egg production is not well documented.

Group III - Elongate tadpoles with attenuate bodies; tail/body ratio >1.7; denticle formula >2/3; little or no reduction of the internal oral features - i.e., these tadpoles have well-developed gill filters; no cranial musculoskeletal specializations for macrophagy. *Phyllodytes* and *Mantidactylus* species belong in this group, as does *Hyla bromeliacia* and *Hyla dendroscarta*. The New World *Hyla* increase lower denticle rows, the Old World *Phyllodytes* and *Mantidactylus* add upper denticle rows.

Group IV - Shorter tadpoles with stout bodies; tail/body

Table 1. Summary of known obligate phytotelm breeding anurans with free-living larvae. Expanded from Lannoo *et al.* 1987. Does not include direct developers that lay eggs in phytotelmata *(e.g.* some *Eleutherodactylus, Platymantis* and *Philautus).*

Species	Larval Diet	Larval Habitat	References
Bufonidae			
Bufo castaneoticus	detritus	Brazil nut capsules	Caldwell 1993; Caldwell & de Araujo, this volume
Dendrophryniscus brevipollicatus	non feeding	bromeliads	Carvalho 1949
Frostius pernambucensis	non feeding	bromeliads	Cannatella 1986
Mertensophryne micranotis	macrophagy suspected	tree holes, snail shells	Grandison & Ashe 1983
Nectophryne afra	unknown	tree holes	Scheel 1970
Pelophryne brevipes	non feeding	leaf axils of *Pandanus,* tree holes	Inger 1954; Denzer 1994
Stephopaedes anotis	unknown	tree buttresses	Channing 1978
Dendrobatidae			
Colostethus beebei	omnivorous and oophagous	giant bromeliads	Bourne *et al.* 2001
Colostethus bromelicola	unknown	bromeliads	Dixon & Rivero-Blanco 1985
Dendrobates arboreus	unknown	bromeliads	Myers *et al.* 1984
Dendrobates auratus	omnivorous	tree holes, bromeliads, stagnant pools	Silverstone 1975; McDiarmid & Foster 1975
Dendrobates azureus	?	tree holes	Summers *et al.* 1999
Dendrobates biolat	unknown	bamboo	Morales 1992; K. Summers, pers. comm.
Dendrobates castaneoticus	predatory, including cannibalism	Brazil nut capsules	Caldwell & de Araujo 1998, this volume
Dendrobates fantasticus	unknown	bromeliads, leaf axils	K. Summers, pers. comm.
Dendrobates granuliferus	conspecific eggs	bromeliads, leaf axils	van Wijngaarden & Bolanos 1992
Dendrobates histrionicus	conspecific eggs	bromeliads	Zimmermann & Zimmermann 1985; Silverstone 1975
Dendrobates lamasi	unknown	unknown	Morales 1992; Schulte, 1999; K. Summers, pers. comm.
Dendrobates lehmanni	conspecific eggs	bromeliads	Zimmermann & Zimmermann 1985; Silverstone 1975
Dendrobates leucomelas	detritus (possibly cannibalistic in lab)	tree holes and trunks, bromeliads	Zimmermann & Zimmermann 1980
Dendrobates minutus	detritus, cannibalistic	bromeliads	Summers *et al.* 2000
Dendrobates mysteriosus	unknown	bromeliads	Schulte 1986; K. Summers, pers. comm.
Dendrobates pumilio	conspecific eggs	bromeliads, terrestrial aroids	Weygoldt 1980; Starrett 1960
Dendrobates reticulatus	omnivorous (cannibalistic and oophagous in lab)	bromeliads	Zimmermann & Zimmermann 1984
Dendrobates speciosus	conspecific eggs	bromeliads	Jungfer 1985
Dendrobates tinctorius	?	tree holes	Summers *et al.* 1999
Dendrobates truncatus	?	tree holes	Summers *et al.* 1999
Dendrobates vanzolinii	conspecific eggs	tree holes	Caldwell 1997
Dendrobates variabilis	detritus, mosquito larvae, conspecific eggs, cannibalistic	bromeliads, tree holes	K. Summers, pers. comm.
Dendrobates ventrimaculatus	detritus, mosquito larvae, conspecific eggs, cannibalistic	bromeliads, *Heliconia* leaf axils	Zimmermann and Zimmermann 1984; Summers 1999
Phyllobates lugubris	omnivorous in laboratory	tree holes and bromeliads	Silverstone 1976; Zimmermann 1982
Phyllobates vittatus	omnivorous in laboratory	tree holes and bromeliads	Silverstone 1976; Zimmermann 1982
Hylidae			
Agalychnis craspedopus	unknown	tree holes and buttresses	Block *et al.* 2003
Anotheca spinosa	conspecific frog eggs, insect matter	bromeliads, tree holes	Taylor 1954; Duellman 1970; Jungfer 1996
Calyptahyla crucialis	frog eggs (conspecific?)	bromeliads	Dunn 1926
Flectonotus fissilis	non feeding	bromeliads	Duellman & Gray 1983
Flectonotus fitzgeraldi	non feeding	bromeliads, aroids	Kenny 1969
Flectonotus goeldii	non feeding (but see Weygoldt and Silva 1991)	bromeliads	Duellman & Gray 1983
Flectonotus ohausi	non feeding	giant bamboo	Lutz 1954; Duellman & Gray 1983
Flectonotus pygmaeus	non feeding	bromeliads and *Heliconia* axils	Duellman & Maness 1980
Hyla bromeliacia	plant matter and detritus	bromeliads	Stuart 1948; Duellman 1970
Hyla dendroscarta	plant matter and detritus	bromeliads	Taylor 1940; Duellman 1970
Hyla marianae	frog eggs (conspecific?)	bromeliads	Dunn 1926
Hyla picadoi	unknown	bromeliads	Robinson 1977; Duellman 2001
Hyla wilderi	frog eggs (conspecific?)	bromeliads	Dunn 1926
Hyla zetecki	frog eggs (conspecific?)	bromeliads	Dunn 1937; Duellman 2001

Table 1. Continued.

Species	Larval Diet	Larval Habitat	References
Nyctimantis rugiceps	unknown	bamboo stumps and tree holes	Crump 1974
Ololygon perpusilla	detritus	bromeliads	Lutz 1973
Osteocephalus buckleyi	unknown	bromeliads and palm fronds	H. Zimmermann, pers. comm.
Osteocephalus deridens	frog eggs	bromeliads	Jungfer et al. 2000
Osteocephalus oophagus	frog eggs	bromeliads, palm bracts, tree holes	Jungfer & Schiesari 1995
Osteopilus brunneus	conspecific eggs	bromeliads	Dunn 1926; Orton 1944; Lannoo et al. 1987
Phyllodytes acuminatus	unknown	bromeliads	Bokermann 1966
Phyllodytes auratus	carnivorous?	bromeliads	Kenny 1969
Phyllodytes brevirostris	unknown	bromeliads	Peixoto & Cruz 1988
Phyllodytes edelmoi	unknown	bromeliads	Peixoto et al. 2003
Phyllodytes gyrinaethes	frog eggs, detritus	bromeliads	Peixoto et al. 2003
Phyllodytes kautskyi	unknown	bromeliads	Peixoto & Cruz 1988
Phyllodytes luteolus	detritus	bromeliads	Bokermann 1966; Giaretta 1996
Phyllodytes melanomystax	unknown	bromeliads	Caramaschi et al. 1992
Phyllodytes tuberculosus	detritus	bromeliads	Bokermann 1966
Phrynohyas resinfictrix	detritus, conspecific frog eggs	tree holes	Schiesari et al. 1996, 2003
Scinax perpusillus	unknown	bromeliads	de Oliveira, pers. comm.
Hyperoliidae			
Acanthixalus spinosus	algae	tree holes	Savage 1952; Perret 1962, 1966; Inger 1966
Acanthixalus sonjae	detritus, insects, other tadpoles in captivity	tree holes	Rödel et al. 2003, Rödel pers. comm.
Hyperolius acutirostris	unknown	tree holes	Perret 1966
Hyperolius mosaicus	vegetable matter, insect larvae, dead conspecifics	tree holes	Perret 1959, 1966
Leptodactylidae			
Crossodactylodes sp.	detritus	bromeliads	Peixoto 1981, 1983
Mantellidae			
Mantella laevigata	conspecific eggs	bamboo stumps, tree holes	Glaw et al. 2000; Heying 2001; Oetter et al. 2001
Mantidactylus albolineatus	unknown	*Pandanus* leaf axils	Blommers-Schlösser 1979
Mantidactylus bicalcaratus	detritus	*Pandanus* leaf axils	Razarihelisoa 1974; Blommers-Schlösser 1979; Lehtinen 2004
Mantidactylus flavobrunneus	unknown	*Pandanus* leaf axils	Blommers-Schlösser 1979
Mantidactylus pulcher	unknown	*Pandanus* leaf axils	Blommers-Schlösser 1979
Mantidactylus punctatus	detritus	*Pandanus* leaf axils	Blommers-Schlösser 1979; Lehtinen 2004
Mantidactylus sp. nov.	unknown	*Pandanus* leaf axils	Lehtinen, unpublished
Microhylidae			
Anodonthyla boulengeri	non-feeding	tree holes, tree ferns, *Ravenala* axils, bamboo stumps	Blommers-Schlösser 1975; Heying 2001
Anodonthyla nigrigularis	non-feeding	tree holes	Glaw & Vences 1994
Anodonthyla rouxae	unknown	bamboo trunks and *Pandanus* axils	Blommers-Schlösser & Blanc 1991
Chaparina fusca	unknown	tree holes, pools	Brown & Alcala 1983; Parker 1934; Inger 1956
Cophyla phyllodactyla	non feeding	tree holes, bamboo trunks	Glaw & Vences 1994
Hoplophryne rogersi	debris, conspecific egg fragments	bamboo and banana leaves	Noble 1929
Hoplophryne uluguruensis	suspected to be the same as *H. rogersi*	bamboo and banana leaves	Noble 1929
Hypopachus variolosus	filter feeding?	puddles and ponds; tree buttresses (rarely)	McDiarmid & Foster 1975
Kalophrynus pleurostigma	non feeding	holes in logs, *Nepenthes* pitchers, pools	Inger 1966; Lim & Ng 1991
Metaphrynella sundana	unknown?	tree holes	Boulenger 1912; Inger 1966; Dring 1979
Platypelis barbouri	non feeding	bamboo holes, *Pandanus* leaf axils	Blommers-Schlösser 1975; Glaw & Vences 1994
Platypelis grandis	non feeding	tree holes, bamboo or *Ravenala* leaf axils	Blommers-Schlösser 1975
Plethodontohyla notosticta	non-feeding	tree holes, tree ferns, *Ravenala* axils, bamboo stumps	Blommers-Schlösser 1975; Heying 2001
Ramanella nagaoi	unknown?	tree holes	Manamendra-Arachchi & Pethiyagoda 2001
Ramanella obscura	unknown	water-filled tree roots	Manamendra-Arachchi & Pethiyagoda 2001

Table 1. Continued.

Species	Larval Diet	Larval Habitat	References
Ramanella triangularis	unknown	tree holes	Inger *et al.* 1985
Syncope antenori	non feeding	bromeliads	Krugel & Richter 1995
Petropedetidae			
Phrynobatrachus guineensis	conspecific eggs, detritus	tree holes, fruit capsules	Rödel 1998; Rödel *et al.*, this volume
Rhacophoridae			
Chirixalus eiffingeri	conspecific eggs	bamboo stumps	Ueda 1986; Kam *et al.* 1996
Nyctixalus pictus	unknown	tree holes	Wassersug *et al.* 1981
Nyctixalus spinosus	unknown	tree holes	Taylor 1962; Alcala & Brown 1982
Philautus sp.	frog eggs	tree holes	Wassersug *et al.* 1981
Rhacophorus cf. *harrissoni*	generalized diet	tree holes	Wassersug *et al.* 1981
Theloderma gordoni	unknown	tree holes	Taylor 1962
Theloderma horridum	unknown	tree holes and buttresses	Boulenger 1903; Wassersug *et al.* 1981
Theloderma leporosa	unknown	tree holes and buttresses	Boulenger 1912
Theloderma stellatum	particulate matter, detritus	tree holes	Wassersug *et al.* 1981

ratios <2, denticle formula greater than 2/3; little or no reduction of internal oral features associated with macrophagy; gill filters and gill filaments normal to greater than normal density; darkly pigmented. Examples include several rhacophorids (*Rhacophorus*, *Theloderma*) and the hylid *Phrynohyas resinifictrix*.

Group V - *Mertensophryne micranotis* with its strange crown and lack of early lung development, is so different from other arboreal tadpoles that it defines its own group.

PRESENT RESEARCH AND ADVANCES

At the present time, we are aware of no fewer than 102 species (in 44 genera and 9 families) that use phytotelmata for reproductive activities (Table 1). Unlike *O. brunneus*, however, very few of these species have been studied in any detail. For example, Peixoto *et al.* (2003) recently described a new bromeliad-breeding hylid from Brazil (*Phyllodytes gyrinaethes*) with extremely unusual tadpoles. These larvae are transparent, have an arched lobe of the dorsal fin covered with tubercles, and a highly modified oral disc that may have some suctorial function. This bizarre larval morphology does not fit into any current classification of larval types that inhabit phytotelmata (Lannoo *et al.*, 1987; McDiarmid & Altig, 1999). What is the function of these strange structures and how does it relate (if at all) to their bromeliad habitat? These questions currently have no answers, but it exemplifies the paucity of data on most phytotelm-breeding frogs. The papers in this volume provide a leap forward in this respect, providing detailed data and careful investigations of several poorly known species.

In perhaps the most complete study of the natural history of any African anuran, Rödel *et al.* provide detailed and long-term data on the natural history of a recently discovered tree hole breeder from Côte d'Ivoire (*Phyrnobatrachus guineensis*). This species uses small tree holes (as little as 3.7 ml volume) and develops rapidly, completing metamorphosis in as little as 15 days. Unlike many phytotelm-breeding frogs, this species exhibits no parental care. A similar life history strategy is used by *Bufo castaneoticus* from the Brazilian Amazon. Using a series of experiments, Caldwell and de Araújo show that the larvae of this species develop rapidly in water-filled Brazil nut capsules to avoid predation by insects and the sympatric *Dendrobates castaneoticus*. Caldwell and de Araújo suggest that this predatory behavior in *D. castaneoticus* tadpoles provides access to energy rich food as well as removing competitors and predators from the water body. Like *Phyrnobatrachus guineensis*, however, neither *Bufo castaneoticus* nor *Dendrobates castaneoticus* exhibit any parental care.

Even though frogs that breed in phytotelmata only represent around 2 % of currently recognized anuran taxa, at least 40 % of these species have some form of parental care. This is a much higher proportion than the estimated 10 % of all frogs that exhibit parental care (McDiarmid, 1978). Phytotelm breeders

also exhibit a wide variety of parental care types. Of the ten types defined by Lehtinen and Nussbaum (2003), phytotelm breeders exhibit at least six (nest construction, egg attendance, egg transport, tadpole attendance, tadpole transport, and tadpole feeding).

The preponderance of parental care in phytotelm-breeders clearly relates to the constraints of these environments. Tadpole feeding, for example, has evolved numerous times in several different lineages, probably in response to food limitation. Provisioning of tadpoles with eggs is known from numerous species of *Dendrobates* and, in a wide-ranging review, Summers & McKeon summarize the work that has been done on phytotelm-breeding dendrobatids. Summers & McKeon also present original data on developmental plasticity, larval ecology, and the factors influencing egg, tadpole and clutch size in this lineage. Tadpole feeding also occurs in the Old World equivalent of dendrobatids, the *Mantella* from Madagascar. In her paper, Heying experimentally demonstrates that oviposition sites (tree holes or bamboo stumps, in this case) are a limiting resource for *Mantella laevigata*. Because of the unique resource that phytotelmata provide to phytotelm-breeding anurans, the demonstrated limitation of population size by oviposition site availability may be a general result in many systems.

A question that has long intrigued herpetologists is how phytotelm breeding evolved in the first place. That is, what was the origin of this trait? Phylogenetic information indicates that the ancestors of phytotelm-breeding dendrobatids were likely stream breeders (see Caldwell & de Araújo; Summers & McKeon, this volume). The phylogeny of Lehtinen *et al.* suggests that in the mantelline frogs of Madagascar, phytotelm breeding evolved twice independently, but both times from pond-breeding ancestors. This analysis also suggests that facultative phytotelm dwelling was a precursor to obligate phytotelm breeding in one lineage. A similar transitional step to obligate phytotelm breeding is hinted at by Caldwell and de Araújo, who observed facultative egg deposition in Brazil nut capsules by two basal, stream-breeding dendrobatids.

These studies (and others, see especially Krügel & Richter (1995); Jungfer (1996); Kam *et al.* (2001); Lardner & bin Lakim (2002), and Proehl (2002)) have used observational, experimental and phylogenetic approaches to address a wide variety of questions. The research presented in this collection of papers has contributed to an improved understanding of the ecological and evolutionary forces acting on phytotelm-breeding anurans and exemplifies the variety of research that is currently underway on anurans that breed in these habitats. Of course, many questions remain unanswered. In the next section, we discuss the prospect of future research to address some of these remaining problems.

FUTURE RESEARCH DIRECTIONS

Given the nature of these micro-aquatic habitats, we expect increased use of phytotelm systems to study the ecology and evolutionary biology of anurans. An obvious advantage of working in phytotelmata is that each phytotelm is a self-contained microcosm whose parameters (biotic, physico-chemical) can be precisely determined. This is particularly advantageous in studies of tadpoles.

Phytotelmata are the simplest aquatic ecosystem used by vertebrates. Yet paradoxically, the diversity of tadpoles from these small aquatic habitats rivals, if not exceeds, the diversity of all tadpoles found in larger pools and ponds. Consider the following. Even within the same genus, phytotelm-dwelling tadpoles can be unusually long and thin (*e.g.*, *Hyla dendroscarta, Hyla bromeliacia*), or fat and wide (*e.g.*, *Hyla picadoi, Hyla zetecki*). They can have no keratinized mouthparts (*e.g.*, microhylids, such as *Ramanella triangularis* and *Hoplophryne rogersi*, and several *Hyla*). They can have the typical denticle pattern of pond tadpoles, namely two denticle rows above and three below the opening of the mouth (*e.g.*, *Crossodactylodes bokermanni, Colostethus bromelicola, Phrynohyas resinifictrix*). But they can also have more exotic dental formulas, with both supernumerary (such as 5/3 in certain *Nyctixalus* spp.) or abbreviated rows (such as 1/1 in various *Dendrobates* spp.).

Because of the relative simplicity of the environments in which these tadpoles live, we predict that our understanding of the functional (eco)morphology of these tadpoles will advance faster than for pond larvae. Functional morphologists understand the design of an organism when they can correctly predict its morphology from its ecology, or vice versa. We already know, for example, more about oral adaptations for predation and oophagy in phytotelm tadpoles, such as those of *Anotheca* and *Osteopilus brunneus* (Wassersug & Hoff, 1979; Lannoo *et al.*, 1987), than about food processing and ingestion in classical, pond-dwelling carnivorous tadpoles, such as those of *Spea* and *Ceratophrys*. We also know that the more attenuate phytotelm-dwelling tadpoles are designed for insinuating themselves in narrow clefts of leaf axils. In contrast, more robust phytotelm-dwelling tadpoles are more often found in open tree holes and other single arboreal tanks.

Phytotelm tadpoles that live in tanks (as opposed to leaf axils) can, in theory, be observed easier than many tadpoles that live in ponds. Thus, despite the fact that many phytotelm sites may be initially difficult to locate, once found, they can provide excellent observatories for tadpole behavior. It is thus not surprising that some of the most complex and elegant behaviors that any tadpoles have shown in relation to both their parents and predators have been documented in phytotelmata. Phytotelm systems are likely to continue to be exceptional in advancing our knowledge of tadpole behavior.

The promise of phytotelm systems to contribute disproportionately to our understanding of anuran behavioral ecology extends to adults as well. Many adult frogs that use phytotelms are conspicuous and diurnal, particularly those in the families Dendrobatidae and Mantellidae. Because of

their conspicuousness, herpetologists have a better chance of tracking their movements and activities.

With intense direct field observation, we may soon find out how these frogs locate phytotelmata in the first place. Do they hunt for them randomly, or do they have a more structured search pattern? Is vision the primary sensory modality used, or do they employ other sensory systems, such as olfaction? Can they sense a humidity gradient with enough precision that they can use it to locate a suitable phytotelm? If the frogs have hyper-sensitive humidity receptors, where are they located on the frog's body? These are all tractable questions for which it is reasonable to expect answers in the near future. Whatever information we glean on how phytotelm-dependent species find their breeding sites, we suspect it will be applicable to more generalized aquatic breeding frogs. Simply because of the visibility of the adults and the discrete nature of phytotelmata, phytotelm-breeding species are a choice model system for addressing these questions in the field.

In a similar vein, we know very little about chemical communication in anurans, other than that it does occur. There is, however, good evidence of intraspecific chemical signaling between phytotelm tadpoles and their mothers in at least one species that actively feeds its tadpoles (Kam & Chen, 2002). Once again, because of the discrete nature and small size of phytotelmata, phytotelm anurans are good model organisms for identifying the molecules involved. We expect these molecules to be identified in the near future. This would then open the way to more reductionist questions about, among other things, the neurobiology of parent-offspring recognition systems and the evolution of the signaling molecules.

Similarly, as several of the contributors to this collection of papers have demonstrated, direct interspecific interactions of phytotelm tadpoles—both those that pertain to competition, as well as predation—are accessible in many phytotelmata. Since phytotelmata are mesocosm systems that are natural and complete, they can inform us about tadpole community ecology with far greater realism than what can be achieved with the cattle tanks commonly used in empirical studies on anuran community ecology.

Because of small clutch sizes, and the limited number of adults in many phytotelm systems, a plethora of classic questions in population biology may be more easily answered with anurans that use phytotelmata than more typical pond-breeding frogs. For example, what limits dispersal and gene flow in these anurans? Several of the papers in the present collection demonstrate the potential of phytotelm-breeding species to answer these questions. Despite their largely tropical occurrence, many phytotelm-using anurans are more amenable to direct studies on population structure and gene flow than common pond species. Indeed such studies are already underway.

In trying to map out future directions for phytotelm research, we are neither prescribing to others what research they should do nor attempting to prognosticate much beyond the obvious.

What *is* obvious to us is that interest in these anurans has been and will continue to be far greater than one would expect from the relatively limited number of species with arboreal tadpoles. Simply stated, these frogs are not just fascinating in their own right; they are ideal for answering a multitude of questions applicable to the multitude of more generalized anurans.

ACKNOWLEDGMENTS

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HISTORICAL AND ECOLOGICAL FACTORS INFLUENCE SURVIVORSHIP IN TWO CLADES OF PHYTOTELM-BREEDING FROGS (ANURA: BUFONIDAE, DENDROBATIDAE)

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ABSTRACT

Throughout Amazonia, Brazil nut trees (Lecythidaceae, *Bertholletia excelsa*) produce a grapefruit-sized fruit with a thick, woody pericarp that is functionally indehiscent; each fruit contains 25 or more seeds (Brazil nuts). After falling to the ground, the fruit capsules are chewed open and emptied of their seeds by agoutis (genus *Dasyprocta*). The empty capsules remain on the forest floor and fill with rainwater. Five Amazonian frog species in two clades, Dendrobatidae and Bufonidae, and two insects with predaceous larvae use Brazil nut capsules for some aspects of reproduction. These small microhabitats lack some kinds of predators (fish) but have others (insects) and can have limited food and low oxygen levels. Interactions among tadpoles and insect larvae and the possible effects of food limitation and anoxia were studied at three sites in Brazil. *Bufo castaneoticus* deposits clutches of eggs that are small compared to most other species in the genus (mean number of eggs: 178 at one locality and 234 at another locality). Survivorship of eggs of *Bufo castaneoticus* at all sites was low. Mean volume of water in the capsules at two localities was 110.9 ml and 132.4 ml; thus, eggs and larvae are crowded, presumably leading to anoxia, especially in the absence of rainfall. An experiment in which tadpoles were raised with and without food revealed that metamorphosis does not occur in unfed tadpoles; thus, food limitation may decrease growth and survivorship. Damselfly larvae occur significantly more frequently with tadpoles of *Bufo* than in all capsules in the samples. These factors appear to provide a competitive release for *Bufo* tadpoles; reduction of tadpole density may increase the probability that some individuals will survive. If they are the first colonizers, the predaceous tadpoles of *Dendrobates* can eliminate predators from the capsules. More basal clades of dendrobatids have detritivorous tadpoles that are not capable of eliminating predators from the capsules. Although they primarily use small forest pools and stream edges for tadpole deposition, tadpoles of *Allobates femoralis* and *Colostethus* sp. were transported occasionally to Brazil nut capsules, where their survivorship was low compared to *Dendrobates*. The propensity of individuals in basal clades for depositing some tadpoles in phytotelmata may have led in part to the evolution of use of phytotelmata by the derived *Dendrobates* once a predaceous tadpole evolved. Occasional deposition of tadpoles in phytotelmata by basal dendrobatids may represent a transitional step from obligate tadpole deposition in forest streams or pools to facultative phytotelm tadpole deposition to obligate phytotelm deposition (*Dendrobates*).

Key words: Bufonidae, *Bufo, B. castaneoticus*, Dendrobatidae, *Allobates, A. femoralis, Dendrobates, D. castaneoticus, D. quinquevittatus, Epipedobates, Colostethus*, phytotelmata, Brazil nut capsules

INTRODUCTION

The earliest frogs utilized pond-type bodies of water for breeding (Duellman & Trueb, 1986). As is typical today, these sites may have contained large numbers of predators that led in part to the evolution of terrestrial modes of breeding to avoid predation of eggs and larvae. Evidence for the role of predation as a major selective force for deposition of eggs on land was provided by Magnusson & Hero (1991). In the present-day temperate zone, most frogs still breed in ponds, but seasonality buffers predation to some extent. Temporary ponds dry out, and many species of frogs breed early in advance of insects, thus avoiding larval insect predation (Skelly, 1996). In the tropics, a more equitable climate may have contributed to diversification of frogs by allowing use of water bodies other than ponds. Tropical species within many clades use various types of phytotelmata, or plant-held waters (Fish, 1983; Schiesari *et al.*, 1996; Jungfer & Weygoldt, 1999). Examples of these microhabitats include tree holes, vine holes, bromeliad tanks, leaf axils of various herbaceous plants, and husks of various types of fruit that fall to the ground and hold water.

These phytotelmata, while generally providing safety from some kinds of predators such as fish, present other kinds of challenges. For example, certain insects with predaceous larvae have evolved to use these container habitats for egg deposition; these larvae readily capture small tadpoles. Food resources are limited in these small microhabitats. In some, especially small vine and tree holes and fallen fruit husks, the absence of light precludes algal growth and detritus may be limited or lacking in nutrients. In addition, the amount of water held in some types of phytotelmata is generally small, so oxygen depletion can be a problem or desiccation can occur if rainfall is insufficient.

Brazil nut capsules provide a common type of phytotelmata throughout most of Amazonia (Mori, 1992). Brazil nut trees (Lecythidaceae, *Bertholletia excelsa*) occur in stands of 100 or more trees in terra firme forest throughout the region (Mori & Prance, 1990). The capsular fruit has a thick, woody pericarp that is functionally indehiscent; each fruit contains 25 or more seeds (Brazil nuts). After falling to the ground, capsules are chewed open by agoutis (genus *Dasyprocta*); the seeds are eaten or cached by these large rodents. The empty capsules remain on the forest floor and fill with rainwater, where they are used

during some phases of reproduction by several species of frogs in the clade Dendrobatidae and one species in Bufonidae. In addition, a giant damselfly (Pseudostigmatidae, *Microstigma anomalum*) and a mosquito in the genus *Toxorhynchites* deposit eggs in the Brazil nut capsules and have predaceous larvae (Steffan & Evenhuis, 1981; Corbet, 1983; Lounibos *et al.*, 1987; Caldwell, 1993).

Within Dendrobatidae, nearly all species are diurnal, deposit terrestrial eggs, and transport tadpoles on the parent's back to a suitable aquatic site for the remainder of the tadpoles' development (Zug *et al.*, 2001). Within the most derived dendrobatids (the genus *Dendrobates*), all species use some type of phytotelmata for tadpole deposition (Caldwell & Araújo, 1998). Some examples of types of phytotelmata used include relatively large tree holes (*Dendrobates auratus,* Summers, 1989), tiny tree or vine holes (*Dendrobates vanzolinii,* Caldwell, 1997; Caldwell & Oliveira, 1999), and plant axils or bromeliad tanks (*Dendrobates pumilio,* Brust, 1993; *D. ventrimaculatus,* Summers & Amos, 1997).

Two species, *Dendrobates castaneoticus* and *D. quinquevittatus,* are known to use Brazil nut capsules as tadpole development sites (Caldwell, 1993; this study). *D. castaneoticus* is restricted in distribution to a small area in the state of Pará, Brazil, and *D. quinquevittatus* occurs in eastern Rondônia (Caldwell & Myers, 1990) and western Amazonas, Brazil (JPC, unpubl. data). Brazil nut capsules appear to be the primary microhabitat used by *D. castaneoticus*. A few tadpoles of this species were found in fallen water-filled palm petioles, but these microhabitats were not common. Tadpoles of *D. quinquevittatus* have been found in various kinds of phytotelmata (Caldwell & Myers, 1990). Tadpoles of both species attain a maximum total length of about 30 mm and metamorphose in about two months.

Within Bufonidae, most species deposit large clutches of eggs in small pools, temporary ponds, or stream edges (Crump, 1989). In contrast, *Bufo castaneoticus* is a phytotelm-breeder, depositing eggs primarily in Brazil nut capsules but occasionally in fallen palm petioles. *Bufo castaneoticus* is a leaf litter frog and does not use arboreal phytotelmata. Tadpoles of this species are small (maximum total length, 12 mm) and time to metamorphosis is relatively brief, about 16–20 days. As currently recognized, this species is widespread from eastern Amazonia in Brazil to Amazonian regions in Peru and Bolivia (de la Riva *et al.*, 2000). Future taxonomic work is needed to determine whether more than one species is involved.

We investigated colonization rates and tadpole–insect interactions in Brazil nut capsules at three localities, two in eastern Brazil and one in western Brazil (Caldwell, 1993; Caldwell & Araújo, 1998). Priority of colonization determined the outcome of interactions between the predaceous tadpoles of *Dendrobates castaneoticus* and predatory insect larvae (Caldwell, 1993), although cannibalism was the primary source of mortality of tadpoles of *D. castaneoticus* (Caldwell & Araújo, 1998). The purpose of this study was to investigate

biotic and abiotic factors that affect survivorship of eggs and tadpoles of *Bufo castaneoticus* and survivorship of tadpoles of *D. quinquevittatus* and *Allobates femoralis*. Specifically we examined the effects of anoxia, desiccation, availability of food, and predation on survivorship of these species.

MATERIALS AND METHODS

Colonization by and interactions among anurans and insects in Brazil nut capsules were studied in two ways: 1) Capsules were located throughout the forest and organisms inhabiting them were examined and identified. No capsule was examined more than once. This procedure was carried out at all three sites and provided a snapshot of co-occurring organisms. 2) At the two eastern sites, the Rio Xingu and Rio Curuá-Una, grids of capsules were established in the forest and organisms were allowed to colonize naturally; capsules were examined every other day for several months. A variation on this method was used at the western site in Rondônia; instead of establishing a grid, capsules were located in the forest, flagged, and examined every four days to study colonization, survivorship, and interactions of the organisms. Data collected on the first day after these capsules were flagged allowed a snapshot comparison with the field-sampled capsules from the two eastern localities.

Organisms in the capsules were examined by pouring the contents of a capsule into a white enamel tray. Tadpoles of *Dendrobates, Bufo castaneoticus*, and *Allobates femoralis* in the capsules were counted. In the field-sampled capsules that were examined only once, tadpoles were preserved in 10% formalin and were later measured and staged according to Gosner (1960). Tadpoles in capsules followed over time were counted and measured nearly each time the capsules were examined. In some cases, newly deposited eggs or developing embryos of *B. castaneoticus* were not disturbed but were counted at a later time to avoid inadvertent mortality. Larvae of the two species of insects were counted and measured in all capsules. A flashlight was used to carefully search for small insects, particularly damselfly larvae that sometimes clung to the inside walls of the capsule. Most of the following measurements were taken on each capsule at all sites: outer circumference at the greatest width of the capsule; major and minor orthogonal axes of the opening; depth of water inside the capsule; volume of water; and pH.

Study areas. We examined Brazil nut capsule systems at three study areas, all lowland tropical forest sites. The hydroperiod for capsules in all three regions is approximately 5 months (length of the rainy season). Rio Xingu (hereafter RX), located in the state of Pará at 3°22′S, 51°51′W, was studied in 1987; Rio Curuá-Una (RCU), also in the state of Pará, located 101 km south and 18 km east of Santarém (3°8′44.3″S, 54°50′32.9″W), was studied in 1995; and Parque Estadual Guajará-Mirim (PEG-M), located at 10°19′17.2″S, 64°33′47.9″W in the state of Rondônia was studied in 1998.

At RX and PEG-M, forests were undisturbed. At RCU, sections of the forest were selectively logged about 8 years previous to the study; however, the actual study site had no evidence of recent logging. Caldwell and Araújo (1998) provided additional details concerning RCU, and Caldwell (1993) provided details of the RX site.

At RX, interactions among *Dendrobates castaneoticus, Bufo castaneoticus,* and the two larval insect predators were studied by establishing a grid of 40 capsules and following them every other day for 52 days from 18 January to 10 March (Caldwell, 1993). One hundred thirty-five field-sampled capsules were located in the forest from 7 Feb to 7 Mar and examined once. Some tadpoles of *Colostethus* were observed in the capsules, but data on this species were not recorded.

At RCU, the same four species co-occurred. A grid of 40 capsules was established and followed every other day for 55 days from 26 February to 21 April. The grid was arranged in five lines, each with eight capsules; the lines were irregular to mimic their natural distribution and also to prevent insects from flying in a straight line and ovipositing in each capsule. Additional details pertaining to the establishment of the RCU grid are given in Caldwell & Araújo (1998). A sample of 101 capsules located at random in the forest was examined from 16 Feb to 15 Apr at this site.

At RCU, we also investigated whether tadpoles of *Bufo castaneoticus* could complete metamorphosis without feeding. The ability to do this would negate the idea that competition for limited food in the capsules was influencing survivorship. We established an experiment in which tadpoles were raised under identical conditions, but some were fed and some were not. We obtained a clutch of 189 eggs deposited the morning of 9 Mar. When the eggs hatched the following evening, we selected 60 that were approximately the same size. Wet mass of 10 of these was obtained by weighing them together; these were not used in the experiments. The remaining 50 tadpoles were established individually in 20 ml of rainwater placed in 30-ml vials; a random numbers table was used to assign tadpoles to the two treatments. Tadpoles were fed and the water was changed every three days; for the first two feeding periods, they were fed Tetra Pelleted Food for Goldfish (0.20 g pellet/vial) and thereafter they were fed with equal amounts of enough leaf litter debris from a nearby pond to cover the bottom of the vial. Metamorphosis was defined as the presence of a tail stub; metamorphs were weighed to the nearest 0.002 g.

At PEG-M, work on the Brazil nut system was carried out for 75 days, from 25 January to 9 April; capsules were examined every fourth day. *Dendrobates quinquevittatus* was moderately common in this area and was the primary anuran that used Brazil nut capsules. *Bufo castaneoticus* occurred within 10–20 km (G.R. Colli and A.A. Garda, pers. comm.) but was not found in the immediate vicinity of the study site. *Allobates femoralis* vocalized in the study area, but only two tadpoles were found in Brazil nut capsules. A single *Colostethus* tadpole (another dendrobatid) was found in a capsule at this site. As at the two sites in eastern Brazil, giant damselfly larvae in the genus *Microstigma* and larvae of the mosquito *Toxorhynchites* were found in Brazil nut capsules.

Seventy-one field-sampled capsules were located in the forest at PEG-M over a period of 13 days from 25 Jan to 6 Feb. Data recorded for these capsules on the first day each capsule was flagged were used as a snapshot to compare with data from the field-sampled capsules at the two eastern sites. The 71 flagged capsules were subsequently examined every fourth day.

Statistical analyses were carried out using Statview (Abacus Concepts, 1992). All means are ± 1 SD. Tadpole size refers to total length (measured from tip of snout to tip of tail).

RESULTS

Water volume in field-sampled capsules was significantly different among the three sites (ANOVA, $F_{2,304} = 20.0$, $p < 0.0001$); volume was smallest at the most eastern site (RX) and largest at the western site (Table 1). Capsules at the site in western Brazil (PEG-M) had a larger circumference than field-sampled capsules at RCU (t-test, $t = -3.8$; $p < 0.0002$; Table 1). pH of capsule water taken at the grid at RCU and field-sampled capsules at RX was acidic; pH of capsule water from these two sites was marginally significantly different (t-test, $t = 2.0$, $p = 0.046$; Table 1).

Males of *Bufo castaneoticus* call from within Brazil nut capsules or while sitting on the capsule to attract a female. Amplexus is axillary (Fig. 1). Egg strings are deposited in the capsule and hatch within two days (Fig. 2). Mean clutch size of five clutches deposited at RX was 178 ± 130.3 eggs (range, 61–387), and of four clutches at RCU was 234.5 ± 101.8 (range, 111–328). Metamorphosis occurred within 16–20 days at RX and within 22–28 days at RCU.

Frogs in the genus *Dendrobates* have small clutch sizes, typically from 2–6 eggs, depending on species (Caldwell & Araújo, 1998). Eggs are deposited in terrestrial sites, where they develop into tadpoles. Tadpoles are then transported singly in most species to some type of phytotelm (Fig. 3). From deposition to metamorphosis required 53.2 days in *D. castaneoticus* (Caldwell & Araújo, 1998). Tadpoles of all species of *Dendrobates* are predaceous. More primitive clades of dendrobatids such as *Colostethus, Allobates*, and *Epipedobates* have larger clutches of eggs (up to 30 in some species), and in these species, the parent transports all tadpoles on its back at one time, usually to small pools or stream edges in the forest. Tadpoles of these species are detritivores.

Field-sampled Brazil Nut Capsules. Dendrobates castaneoticus transported its tadpoles to Brazil nut capsules at both of the eastern study sites (Table 2); this frog was more common at RCU than RX. Its sister species, *D. quinquevittatus,* used the capsules as tadpole nurseries in western Brazil (PEG-M). No other species of *Dendrobates* were present at the three sites. Tadpoles of the dendrobatid *Allobates femoralis* were

Table 1. Physical characteristics of field-sampled Brazil nut capsules selected at random and those used to establish the colonization grid at RCU. Values are means ± 1 SD; ranges are given in parentheses. OA = orthogonal axis.

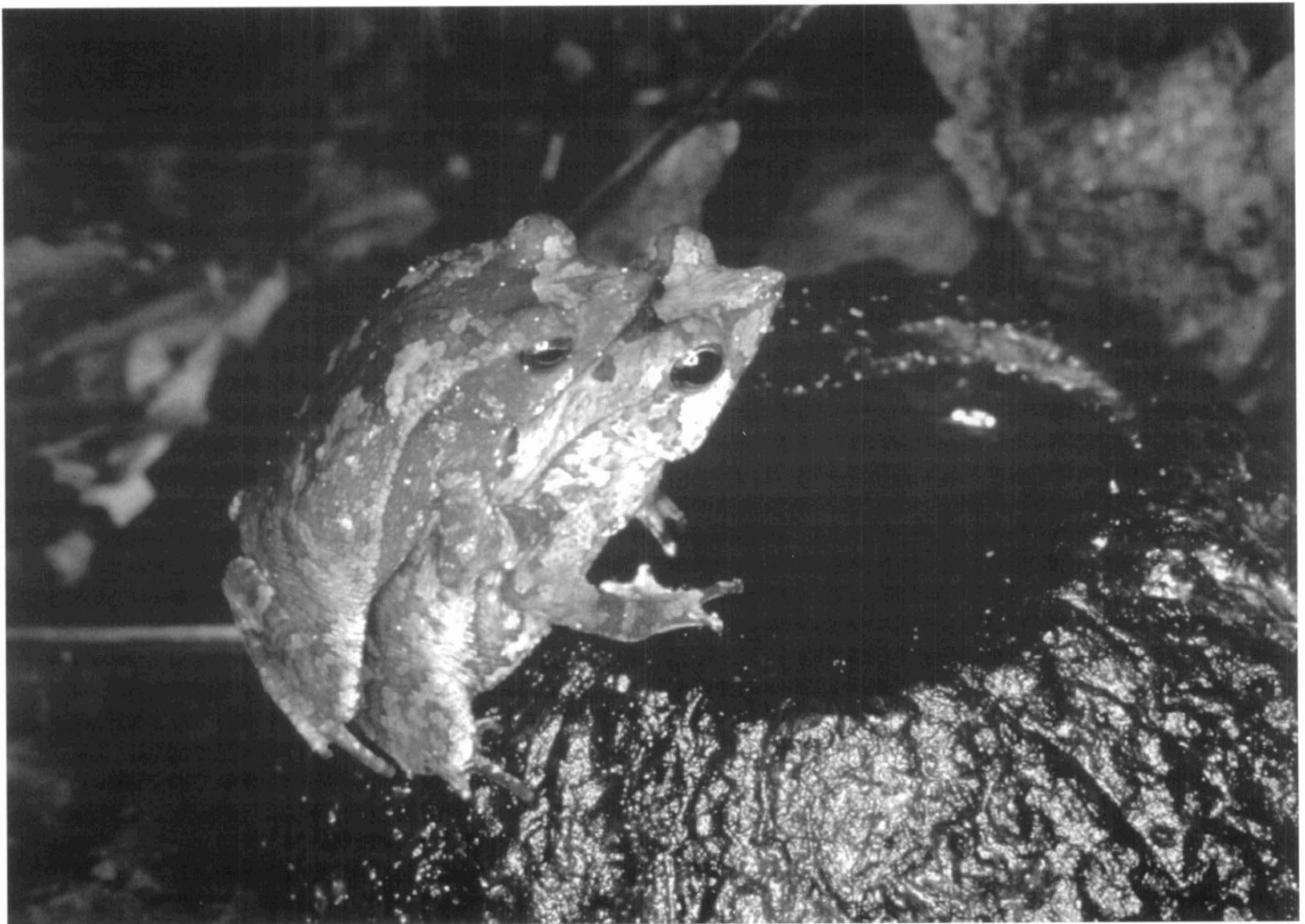|	RX, field n = 135	RCU, field n = 101	RCU, grid n = 40	PEG-M, field n = 71
Water volume (ml)	110.9 ± 55.1 (5–255)	132.4 ± 80.0 (3–324)	206.5 ± 57.9 (85–304)	177.9 ± 87.8 (22–408)
Circumference (cm)	----	33.5 ± 3.7 (25–43)	32.1 ± 2.1* (28–37)	35.7 ± 3.1** (23–41)
Major OA (mm)	45.4 ± 11.2 (25–79)	43.4 ± 7.3 (28–71)	44.9 ± 8.1 (34–73)	49.5 ± 13.5 (29–79)
Minor OA	37.7 ± 10.2 (20–72)	36.4 ± 6.1 (24–56)	39.8 ± 7.8 (29–69)	42.8 ± 13.5 (24–75)
pH	5.4 ± 0.6*** (4.2–6.8)	----	5.6 ± 0.8 (3.9–6.9)	

*n = 37; **n = 56; ***n = 134.

Fig. 1. An amplexing pair of *Bufo castaneoticus* from the Rio Curuá-Una study site. This pair subsequently entered the Brazil nut capsule and deposited eggs there.

Fig. 2. Eggs and tadpoles of Bufo castaneoticus. In this case, egg strings of *Bufo castaneoticus* were deposited in a capsule in which a previous clutch had been deposited.

found infrequently in the capsules at RX and RCU (Table 2). Adults were seen and heard at the western site, but no tadpoles were present during the 13-day period when the capsules were first located. (Subsequently, as the capsules were followed over time, two *A. femoralis* tadpoles were found.)

Damselfly larvae were fairly common and occurred in 18–25% of the capsules at all three sites. Larvae of *Toxorhynchites* were found at all three sites and were always less common in the capsules than damselfly larvae (Table 2). Other species of mosquitoes were commonly present in the capsules, but were not identified (Table 2, but see Caldwell & Araújo, 1998). At any one time, a small percentage of capsules, from 8–14%, at the three sites contained no organisms (Table 2).

Survivorship of eggs and tadpoles of Bufo castaneoticus. Egg survivorship was variable, but overall low. In the two grids where organisms were monitored (RX and RCU), overall

survivorship of a total of 9 clutches with known number of eggs was $25.8 \pm 30.4\%$ (range, 0–87%). In the two sets of field-sampled capsules, all eggs in 4 of 6 capsules found with clutches were dead. The two other capsules contained 147 and 61 live eggs.

Eight clutches of eggs were deposited in capsules on the RCU grid. Three of these clutches were deposited in one capsule (#12) on 4 Mar, 20 Mar, and 13 Apr, and the other 5 clutches were each in a different capsule, deposited on 8 Mar, 13 Mar, 26 Mar, 3 Apr, and 18 Apr. All eggs in two of the 8 clutches, one deposited on 20 Mar in capsule #12 and one deposited on 8 Mar, had 100% mortality.

In capsule 12, 328 eggs were deposited on 4 Mar. Seven days after hatching, 285 tadpoles (86.9%) remained alive and healthy in this capsule; no insects were present at this time. Seven days later, 266 tadpoles (81.1%) remained alive, but
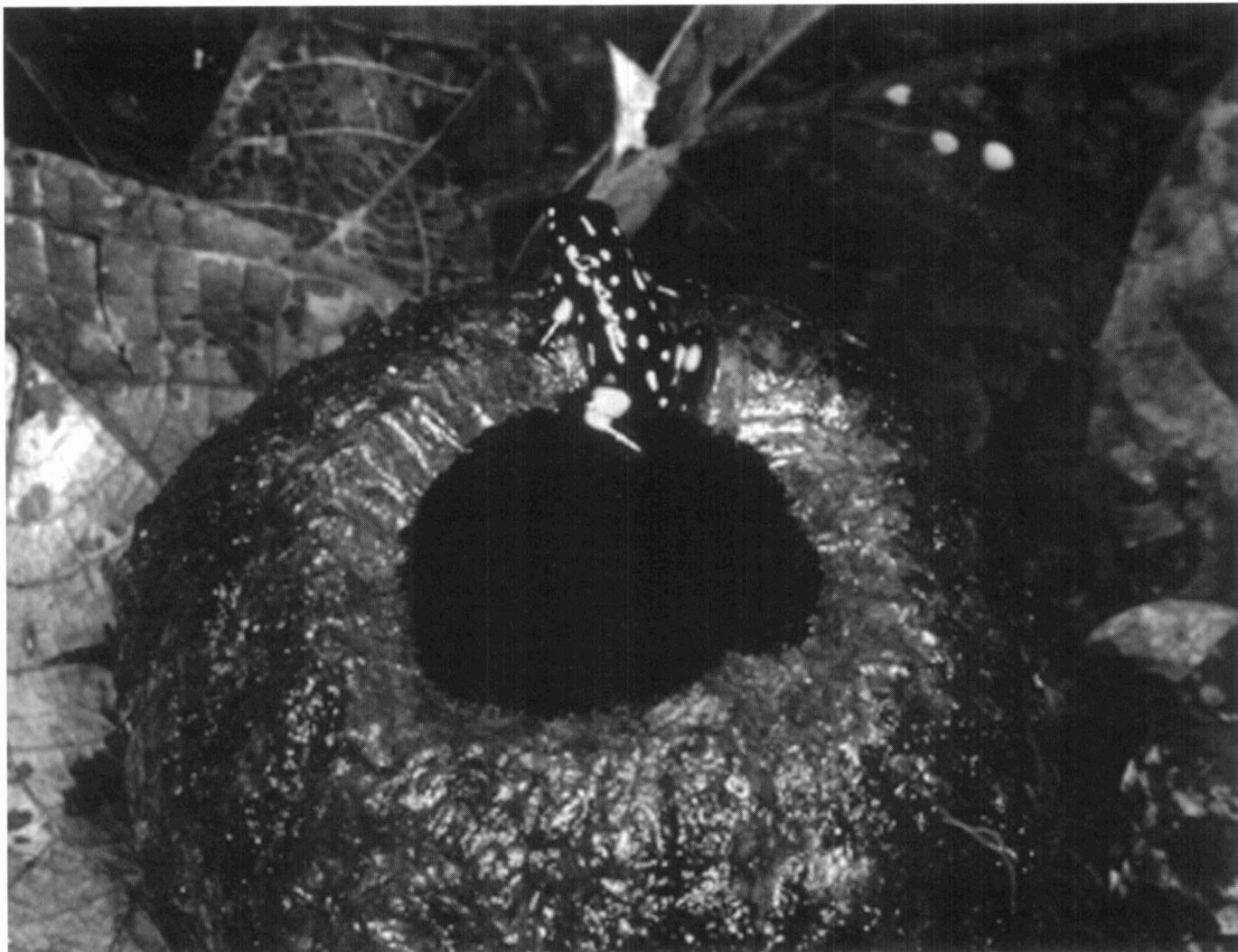
Fig. 3. *Dendrobates castaneoticus* sitting on the edge of a Brazil nut capsule at the Rio Curuá-Una study site. Males of this species transport tadpoles singly to the capsules.

they appeared lethargic; for four days, they moved very little, and many floated upside down. However, a rain of 80 mm revived them, and they continued to remain healthy (Fig. 4). Subsequently, a second clutch deposited on 20 Mar had 100% mortality within 4 days, and a third clutch deposited on 19 Apr had 140 eggs, 131 of which were still alive two days later when the study ended (Fig. 4). A *Toxorhynchites* larva was deposited in this capsule and likely consumed some tadpoles as it grew (Fig. 4). In three other capsules, tadpole survivorship dropped to 25-50% within 4–6 days after eggs were deposited on 14 Mar, 26 Mar, and 3 Apr (Fig. 5).

Metamorphosis was observed in 3 of the 8 clutches deposited on the RCU grid; in 2 other clutches, 100% mortality occurred, and 3 others could not be followed to metamorphosis before the study ended. Metamorphosis began an average of 25.3 ± 3.1 days (range, 22–28 days) after eggs were deposited. In capsule 12, 30.8% of the original clutch metamorphosed during a 9-day period. After this time, 112 tadpoles (34.1%) remained in the capsule and showed no limb development for the next 10 days, at which time the study concluded (Fig. 4).

Results of Feeding Experiment. Fourteen of 25 tadpoles (56%) metamorphosed in the group that was fed, whereas no tadpoles metamorphosed in the unfed group. Metamorphosis of 13 tadpoles occurred from 20–23 days after hatching; the 14th tadpole did not metamorphose until 29 days after hatching. Mean wet mass of the 14 tadpoles at metamorphosis was 0.011 ± 0.002 g (range, 0.008–0.016). Two tadpoles in the group that was fed died during the experiment, compared with 16 in the unfed group. Nine tadpoles (36%) in both groups remained alive but failed to show any limb development after 38 days, when the experiment was terminated. Mass of the 10 newly hatched tadpoles weighed together in the group used to establish the experiment was 0.068 (0.0068/tadpole). Thus, overall increase in size was only 61.8%; most energy appears to be directed toward development.

Observations of tadpoles of Bufo castaneoticus in field-sampled capsules at RCU. Eggs were found in 3 of 101 field-sampled capsules, and 44.9 ± 41.7 tadpoles (range, 11–130)

Table 2. Comparison of organisms inhabiting field-sampled Brazil nut capsules at three sites in Amazonian lowland forest. Values are the number of individuals found in each sample and the percentage of capsules containing that taxon (in parentheses). Percentages do not add to 100% because some species co-occur in a single capsule. For PEG-M, data are from the first time each of the 71 capsules was located (see Methods).

	Pará: RX n = 135	Pará: RCU n = 101	Rondônia: PEG-M n = 71
*Dendrobates**	16 (11.9%)	25 (24.8%)	18 (25.4%)
Bufo castaneoticus	14 (10.4%)	13 (12.9%)	0
Allobates femoralis	4 (2.9%)	20 (8.9%)	0**
Colostethus sp.	-----***	0	1 (1.4%)
Damselfly larvae	25 (18.5%)	25 (24.8%)	17 (23.9%)
Toxorhynchites larvae	13 (9.6%)	3 (3.0%)	9 (12.7%)
Other mosquito larvae	79 (58.5%)	52 (51.5%)	58 (81.7%)
Nothing	19 (14.1%)	11 (10.9%)	6 (8.5%)

D. castaneoticus at RX and RCU; *D. quinquevittatus* at PEG-M.
**Two tadpoles of *A. femoralis* were found as these 71 capsules were followed over time.
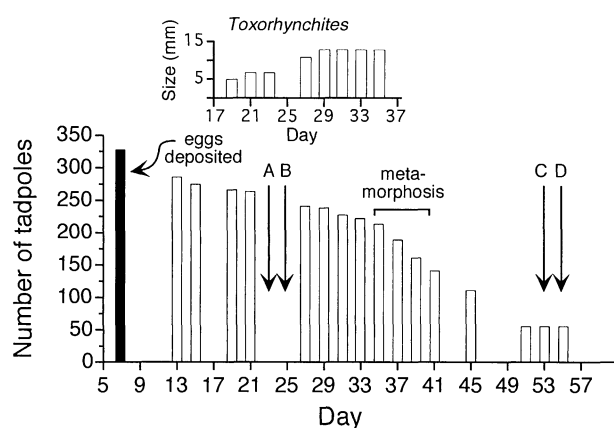***Colostethus* sp. tadpoles were observed in capsules at RX but were not counted.

Fig. 4. History of egg deposition and tadpole development of *Bufo castaneoticus* in Capsule 12 on the grid established at the Rio Curuá-Una study site. Three clutches of eggs were deposited in this capsule. A and B indicate the days of persistence of the second clutch, which had 100% mortality. C and D are days of persistence of the third clutch, which was still present when the study ended at Day 55. Bars refer only to the first clutch deposited. A predaceous *Toxorhynchites* larva was first detected in the capsule on Day 19 and probably accounted for some of the reduction in tadpole numbers before it pupated on Day 37. Metamorphs were detected only from Day 35 to Day 41; after that time, none of the remaining tadpoles developed large hind limbs.

were found in 10 others. Of the 10 capsules with *Bufo* tadpoles, 2 (20%) had no predaceous insects. Seven of the remaining 8 capsules contained damselfly larvae, and 1 capsule contained a *Toxorhynchites* larva and a *Dendrobates* tadpole. Damselfly larvae occurred proportionately more frequently with tadpoles of *B. castaneoticus* (70%) than in all capsules (25 of 101, 24.8%; chi-square, 3.93, *p* = 0.048).

Survivorship of tadpoles of Allobates femoralis on the RCU grid. Relatively few tadpoles of *A. femoralis* were deposited in capsules on the RCU grid; large numbers of tadpoles of this species were found in small pools formed in a nearby logging road in the forest (pers. obs.). A total of 28 tadpoles
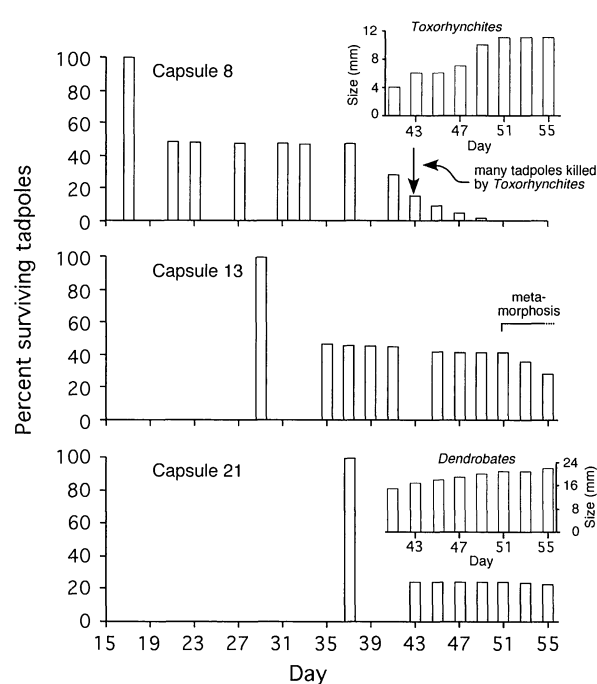

Fig. 5. History of egg deposition and tadpole development in three clutches of *Bufo castaneoticus* on the grid established at the Rio Curuá-Una study site. All three clutches had an initial dieoff of eggs; no predators were present at that time. In Capsule 8, on Day 43, about 20 dead tadpoles, mostly consumed with only skin remaining, were present. After that, tadpole numbers gradually declined, apparently from predation by the *Toxorhynchites* larva. No tadpoles survived to metamorphosis. In Capsule 13, after the initial dieoff of eggs, tadpoles survived and began to metamorphose, which was ongoing when the study ended. In Capsule 21, a *Dendrobates* tadpole was deposited 4 days after the *Bufo* eggs were deposited; whether this tadpole might have killed or eaten the *Bufo* eggs is unknown. From Day 43 until the end of the study, however, the *Bufo* tadpoles coexisted with the *Dendrobates* tadpole.

were deposited in 6 capsules; 17 were deposited at one time in one capsule, and the other capsules had from 1–3 tadpoles
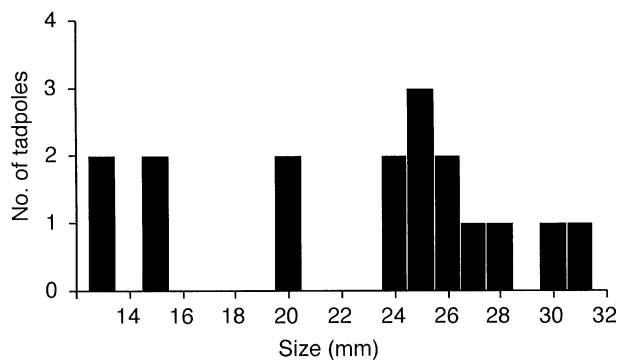
Fig. 6. Size distribution of tadpoles of *Dendrobates quinquevittatus* at the PEG-M study site in 71 capsules examined from 25 Jan to 6 Feb. No additional tadpoles were deposited in capsules in the study area after 8 Feb.

deposited in them. Of the 28 tadpoles, 2 (7.1%) survived to metamorphosis. Two others remained alive at the end of the study, but neither had grown larger than 17 mm and one was with a damselfly larva; thus, both these tadpoles were considered to be non-survivors. Sources of mortality included: being killed and eaten by tadpoles of *Dendrobates castaneoticus* (12 individuals, 46.2%); being deposited in a capsule with a cohort of large but nonpredaceous mosquito larvae (*Trichoprosopon digitatum,* see Caldwell & Araújo, 1998) and possibly dying from oxygen depletion (7 individuals, 26.9%); being killed by damselfly larvae (3, 11.5%); desiccation of capsule water (3, 11.5%); and failure to grow (1, 3.9%). The two tadpoles that survived were in the same capsule; they grew steadily and on the 12th day after they had been deposited, a clutch of *Bufo castaneoticus* eggs was deposited in the same capsule. These two tadpoles, similar in size and color to *Bufo* tadpoles, continued to coexist with the *Bufo* tadpoles and grow.

Observations of tadpoles of Allobates femoralis in field-sampled capsules at RCU. A total of 20 tadpoles were found in field-sampled capsules. From 1–4 tadpoles were found in 9 capsules; thus, 8.9% of the capsules contained tadpoles of *A. femoralis.* Ten of these individuals (50%) were in capsules without insect larvae and thus were not in immediate danger of predation. Of the remaining 10 tadpoles, 7 were in capsules with damselfly larvae; thus, their survivorship was unlikely. The remaining three were in two capsules with 19 and 130 *Bufo* tadpoles; both these capsules contained predators: a *Toxorhynchites* larva in one and 3 damselfly larvae in the other. These *A. femoralis* tadpoles could have survived, depending on whether they could remain inconspicuous among the *Bufo* tadpoles.

Survivorship of dendrobatid tadpoles at PEG-M. Eighteen tadpoles of *Dendrobates quinquevittatus* were found individually in 18 of 71 capsules first located from 25 Jan to 6 Feb. Of these, one was dead when found, apparently from desiccation, and could not be measured. The remaining 17 tadpoles ranged in size from 13 to 31 mm in total length (Fig. 6). Subsequently, 5 additional tadpoles were deposited in the capsules from 4 to 8 Feb; four of these newly deposited

tadpoles were 12.5 ± 1.0 mm in total length (range, 11–13 mm). No additional tadpoles were deposited in any of the 71 capsules after 8 Feb. In two of the five newly deposited tadpoles (40%), a smaller tadpole was deposited in a capsule with a larger tadpole.

Of the 23 tadpoles, 16 (69.6%) reached metamorphosis. Two of the remaining seven (28.6%; both 13 mm in total length) were killed by larger tadpoles (16 and 26 mm total length) already present in the capsules; one (14.3%) was killed by a 19-mm damselfly larvae already present in the capsule; two died when capsules dried out; one was missing from its capsule; and one was still alive at the end of the study, but had failed to grow and most likely did not survive the approaching dry season.

The established *Dendrobates quinquevittatus* tadpoles consumed newly hatched damselfly larvae that appeared in their capsules. Small damselfly larvae found in capsules with *D. quinquevittatus* averaged 3.5 ± 1.9 mm in length (range, 1–5 mm; n = 4); these damselflies persisted with *D. quinquevittatus* tadpoles for 4.5 ± 4.0 days (range, 1–8 days). In contrast, newly hatched damselfly larvae in capsules without tadpoles attained an average size of 14.2 ± 7.1 mm in length (range, 1–23 mm; n = 30) and survived for 41.6 ± 25.6 days (range, 1–75 days). Many of the latter damselfly larvae had not transformed by the end of the study.

Development of tadpoles of *Dendrobates quinquevittatus* to stage 42 in the capsules required 55.3 ± 3.2 days (range, 53–59 days). Based on data from *D. castaneoticus,* an additional time of 5.5 days was required for completion of metamorphosis (Caldwell & Araújo, 1998). Using this figure for *D. quinquevittatus,* the estimated time from deposition to metamorphosis was 60.8 days. Maximum mean total length reached prior to metamorphosis was 28.1 ± 2.7 mm (range, 23–31 mm; n = 16).

Two tadpoles of *Allobates femoralis* were found in a capsule at PEG-M on 16 Feb. The capsule contained a 6-mm and a 16-mm damselfly larva. One of the *A. femoralis* tadpoles was dead with a missing tail when found; the other was missing four days later. No other tadpoles of *A. femoralis* were deposited in capsules at PEG-M. A single *Colostethus* tadpole was found in a capsule on 25 Jan; this tadpole metamorphosed on 4 Mar.

DISCUSSION

This study investigates biotic and abiotic factors that have potentially led to the evolution of strategies of survivorship of eggs and tadpoles of *Bufo castaneoticus* and tadpoles of various species of dendrobatids that inhabit Brazil nut capsules at three Amazonian localities where work has been conducted on this system. Specifically, abiotic factors that influence survivorship in this microhabitat include the ability of eggs and tadpoles to withstand low oxygen conditions and some risk of desiccation as the habitat dries toward the end of the wet season. Biotic factors include acquisition of sufficient food resources for

growth and development and avoidance of predation by insect predators that inhabit the capsules and by two of the species of *Dendrobates* tadpoles, which are predaceous and cannibalistic. Examination of these factors in species in the two clades that use Brazil nut capsules revealed that *B. castaneoticus* tadpoles have reduced clutch sizes in comparison to other species in the genus *Bufo,* relieving some stress of anoxia in a restricted environment, and that a rapid time to metamorphosis allows at least some individuals to escape predation by insect larvae that use the capsules. In contrast, *Dendrobates* tadpoles are transported singly to capsules (although adult frogs often transport more than one tadpole to the same capsule) so that anoxia is not a major factor in survivorship. Predation, particularly by larger conspecifics, accounts for most mortality in *Dendrobates*. Potential insect predators are eaten when they are small (i.e., < 7 mm; Caldwell, 1993) by these predaceous tadpoles; however, if the tadpole is deposited in a capsule with a large predator, the tadpole will be eaten.

Survivorship strategies of Bufo castaneoticus. Survivorship of eggs of *Bufo castaneoticus* at all sites was low. A common occurrence was the death of 25-50% of eggs or early embryos in a clutch of *Bufo* eggs. The remaining tadpoles thrived, possibly because of a reduction in competition for oxygen and food resources.

Most species of *Bufo* typically deposit large numbers of very small eggs (Crump, 1989). Compared with most other species in the genus, *B. castaneoticus* has evolved a relatively small clutch size, possibly as an adaptation to the restricted size of its breeding sites. Even so, the amount of water available for development of these eggs is small (mean volume at RX was 110.9 ml and at RCU was 132.4 ml; Table 1) and eggs are crowded, presumably leading to anoxia in some cases. In this study, entire clutches of eggs frequently died; no evidence of predation was obvious in these cases. In one case, all eggs of a large clutch survived, but the tadpoles became lethargic and floated upside down, apparently because of oxygen depletion. These tadpoles do not have developed lungs (JPC, pers. obs.). Wassersug & Seibert (1975) noted that tadpoles of *B. woodhousii,* which also lacked developed lungs, became motionless on their backs and sides under experimental conditions of < 2.0 ppm dissolved oxygen. Tadpoles in this capsule were subsequently revived by a heavy rainstorm. This study was conducted fairly late in the wet season, and rainfall was becoming sporadic. Possibly survivorship of eggs is higher earlier in the rainy season when more frequent rains may keep oxygen levels elevated in the capsules.

Food limitation may be responsible for failure of some tadpoles to grow and eventually metamorphose. *Bufo castaneoticus* tadpoles, which at a body length of about 5 mm when near metamorphosis are among the smallest tadpoles known, are typical detritivores and can apparently survive and grow on the small amount of detritus in the capsules unless large numbers result in competition for food. In addition, most energy is put into development rather than growth, an indication that nutrient requirements are low. However, in one clutch observed through metamorphosis, one-third of the tadpoles failed to develop, an indication that food was limited. *Bufo periglenes*, another species that breeds in a restricted microhabitat, can successfully metamorphose without feeding (Crump, 1989). A laboratory experiment revealed that metamorphosis does not occur in tadpoles of *B. castaneoticus* if food is withheld. Comparison of egg size of these two species revealed that *B. periglenes* is provisioned with more yolk than *B. castaneoticus* (*B. periglenes*, female size, 50–55 mm SVL, egg diameter, 3.0 mm, Crump, 1989; *B. castaneoticus*, female size, 40–51 mm, egg diameter, 1.5 mm, unpubl. data).

Bufo tadpoles were more likely to co-occur with damselfly larvae than with other organisms at the RX study site (Caldwell, 1993) as well as at the RCU study site. A possible reason for this association may be that *Microstigma* eggs are deposited in many capsules but survivorship of damselfly larvae is higher in those capsules with food (i.e., tadpoles). The result of this association may be that as tadpole density decreases, resource availability increases.

A competitive release therefore appears to occur in several ways in *Bufo* tadpoles: anoxia may kill many of the eggs or young embryos; food limitation may prevent growth or development of some tadpoles; or consumption of tadpoles by predaceous insect larvae may reduce tadpole density. Reduced clutch size in *B. castaneoticus* may reflect an evolutionary tradeoff between producing relatively large, competitive offspring (thus reducing clutch size) and continuing to produce enough offspring to allow some to survive in the presence of predictably high predation and possible death due to anoxia or food limitation in a constrained environment.

Dendrobatid survival strategies. Unlike bufonids, which deposit eggs in water, dendrobatids deposit eggs on land and transport their tadpoles to aquatic sites to complete their development. Tadpoles are transported singly (*Dendrobates castaneoticus* and *D. quinquevittatus*) or in small numbers (*Allobates femoralis* and *Colostethus*). Oxygen deprivation is unlikely to be a cause of mortality in these species for this reason, unless a capsule is partially tipped over or begins to leak, which are rare occurrences. Desiccation can occur as the end of the rainy season approaches, although both *D. castaneoticus* (Caldwell & Aráujo, 1998) and *D. quinquevittatus* ceased transporting tadpoles abruptly near the end of the rainy season.

Food limitation may be a cause of failure to grow in some individuals of *Dendrobates*. Caldwell & Araújo (1998) showed that tadpoles of *D. castaneoticus* supplied with more food grew faster, although only one tadpole of the 42 followed in that study and one in the study on *D. quinquevittatus* became obviously emaciated. Mosquito larvae, which form one source of food for *Dendrobates* tadpoles, are deposited throughout the hydroperiod of the capsules and were present in 52%–82% of field-sampled capsules at the three study sites (Table 2).

Observations on survivorship of tadpoles of *Dendrobates*

quinquevittatus in capsules in western Brazil compared with those of *D. castaneoticus* in eastern Brazil revealed that cannibalism of younger tadpoles by older ones is a common cause of mortality in both species. Although predatory behavior allows *Dendrobates* tadpoles to rid their containers of potential larval insect predators, a tradeoff is that young tadpoles deposited in a capsule with older ones are frequently cannibalized (Caldwell & Araújo, 1998). Mortality due to cannibalism was lower in tadpoles of *D. quinquevittatus* than those of *D. castaneoticus*, possibly because the end of the wet season was approaching and fewer young tadpoles were being deposited. Caldwell (1993) showed that the first colonizer (whether insect larva or tadpole) typically became established in the capsule. Thus, tadpoles that were already established by the beginning of the work at PEG-M were likely to survive. Data on timing of deposition of tadpoles of *D. castaneoticus* also revealed a sharp decrease as the dry season approached (Caldwell & Araújo, 1998).

This study reveals that both *Allobates femoralis* and *Colostethus* sp. occasionally deposit tadpoles in Brazil nut capsules. Survivorship of *A. femoralis* in this microhabitat is very low compared to that of *Dendrobates*. Tadpoles of *Colostethus* and *A. femoralis* are not predaceous and have no mechanism for removing potential predators from the capsules, in contrast to *Dendrobates*. Survival of *A. femoralis* tadpoles appears to occur in two situations. Tadpoles may be deposited by chance in a capsule that does not have predators, or they may be deposited in a capsule that has a clutch of tadpoles of *Bufo castaneoticus*. Tadpoles of *A. femoralis* and *B. castaneoticus* are similar in size and light brown coloration. Typically only a few *A. femoralis* are deposited in a capsule by the parent frog, and these tadpoles may masquerade among the *Bufo* tadpoles; even if a predator is present, an individual tadpole's chance of being captured are decreased. The same scenario may apply to some species of *Colostethus*.

Recent phylogenies of dendrobatid frogs show that *Colostethus* and *Allobates femoralis* are basal members of the dendrobatid clade and that *Dendrobates* is derived in comparison (Clough & Summers, 2000; Vences et al., 2000; Symula et al., 2003). *Dendrobates* has evolved a suite of characteristics that differ from *Colostethus, Allobates,* and *Epipedobates*. For example, they deposit tadpoles in phytotelmata of various types, whereas the basal clades typically use small pools or stream edges in the forest. From an evolutionary point of view, the occasional use of phytotelmata by individuals in some species in basal clades of dendrobatids indicates that early dendrobatids had already evolved a propensity for this behavior. This tendency may represent a transitional step in dendrobatids from obligate tadpole deposition in forest streams or pools to facultative phytotelm tadpole deposition (*Colostethus* sp., *A. femoralis*) to obligate phytotelm deposition (*Dendrobates*).

Being predaceous has other advantages for tadpoles of *Dendrobates* in a food-limited environment. About 60–80% of all capsules examined at all study sites contained small mosquito larvae of various sizes that provided a food source for the tadpoles. One species of mosquito that has larger (and therefore more energy-rich) larvae has an affinity for depositing eggs in newly opened capsules (Lounibos & Machado-Allison, 1983; Caldwell & Araújo, 1998). Tadpoles deposited in those capsules had a growth advantage. In extreme cases, some types of phytotelmata have no food; for example, no mosquitoes breed in the tiny vine holes used by *D. vanzolinii* for egg and tadpole deposition in western Brazil. In this species and several others, females have evolved the ability to provide trophic eggs for the tadpoles (Caldwell & Oliveira, 1999; Summers et al., 1999). The availability of phytotelmata of various kinds, combined with the evolution of diverse forms of parental care, may have contributed in part to the evolution of the diversity of species in *Dendrobates*.

To date, the only two species of *Dendrobates* known to use Brazil nut capsules for tadpole deposition are *D. castaneoticus* and *D. quinquevittatus*. Both of these species have limited distributions (Caldwell & Myers, 1990); thus, this component of the system is absent in much of the Amazon region. *Bufo castaneoticus* is locally common throughout the Amazon region and is known to use Brazil nut capsules from Bolivia to eastern Brazil (de la Riva et al., 2000). The interaction between *Bufo* tadpoles and *Dendrobates* tadpoles remains unknown. In an experimental situation, *Dendrobates* tadpoles killed some *Bufo* tadpoles (Caldwell, 1993); however, at RCU (this study), *Dendrobates* and *Bufo* tadpoles coexisted under field conditions. Additional work will be necessary to determine whether tadpoles of *B. castaneoticus* are toxic and possibly unpalatable to *Dendrobates* tadpoles.

In summary, species in the two clades of anurans that breed in Brazil nut capsules have fundamental differences in life history traits that influence their responses to this predator-rich, frequently anoxic and low-nutrient habitat. *Bufo castaneoticus* deposits clutches of around 200 eggs directly into the capsules, whereas dendrobatids deposit eggs in terrestrial sites and transport their tadpoles either singly *(Dendrobates)* or in small groups (< 30, *Allobates, Colostethus*). Some tadpoles of *Bufo* escape predation because of saturation of the environment by relatively large numbers of individuals, but these large numbers mean that problems with anoxia and food limitation can cause mortality. In contrast, tadpoles of dendrobatids are at low density within a capsule and oxygen stress is lower or nonexistent for them. Within dendrobatids, predation causes higher mortality in tadpoles of *Allobates* than in *Dendrobates*; the former are detritivores whereas tadpoles of *Dendrobates* are predaceous and can eliminate small predators from their environment. However, a tradeoff exists in that predatory tadpoles also consume a large number of smaller conspecifics (Caldwell & Araújo, 1998).

Additional work on this system should investigate aspects of life history traits of *Bufo castaneoticus* compared with closely related species. For example, the idea that clutch size is reduced in this species (and others such as *B. periglenes* that

breed in restricted habitats) could be examined in a comparative framework. Oxygen levels throughout the capsule hydroperiod should be obtained and the sensitivity of *B. castaneoticus* eggs and tadpoles to anoxia could be experimentally determined. Egg survivorship in *B. castaneoticus* could be higher when rainfall is more frequent at the beginning of the rainy season. Whether eggs and tadpoles of this species have a higher tolerance for anoxia than eggs and tadpoles of other species of *Bufo* could be examined. *Bufo castaneoticus* may have oviposition strategies that allow avoidance of predator-rich or anoxic sites. Regarding dendrobatids that use this system, experiments on survivorship of *Allobates femoralis* or *Colostethus* tadpoles masquerading in the presence of *B. castaneoticus* tadpoles should be conducted; predation may be lower in these individuals than in those in a capsule without *Bufo* tadpoles. Seasonality may play a role in survivorship in *Dendrobates* tadpoles; cannibalism may be higher in the early part of the rainy season when more tadpoles are being deposited. This system is simple enough and capsules are abundant enough in many places that interactions can be studied in detail, and realistic experiments on biotic interactions and the effects of abiotic factors can be conducted using Brazil nut capsules as microcosms.

ACKNOWLEDGMENTS

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REPRODUCTIVE LIMITATION BY OVIPOSITION SITE IN A TREEHOLE BREEDING MADAGASCAN POISON FROG (*MANTELLA LAEVIGATA*)

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ABSTRACT

Oviposition sites are critical to the reproductive success of any egg-laying species, particularly those that do not transport eggs immediately after laying. I subjected *Mantella laevigata*, a Madagascan poison frog, to experimental manipulation to determine whether oviposition sites are limiting for this species. The hypothesis that oviposition sites—water-filled treeholes—are limiting for *M. laevigata* was based on my previous observations that females leave courtships to defend their oviposition sites, males leave courtships to defend their oviposition site-containing territories, and females choose mates based solely on oviposition site quality. I found that oviposition sites are limiting for *M. laevigata*. These results are discussed in light of theoretical predictions of multiple versus single limitation. Given that oviposition sites are the single limiting resource for these frogs, the advantages of being choosy about oviposition sites, and the benefits and risks of using treeholes, are delineated. Benefits include an increased ability to keep track of offspring, which may facilitate the evolution of clutch size reduction, parental care, and nest site defense. Risks primarily involve threats to young, including desiccation, predation, cannibalism and competition.

Keywords: limiting factor, reproductive success, phytotelmata, breeding site, predation, cannibalism, competition, Mantellidae

INTRODUCTION

Limiting resources are those which, if increased, would result in an increase in population size. The limiting element for a population can act in multiple ways, but its final effect is always the same. Adding more of a limiting resource may cause existing individuals to become larger or more fecund, resulting in population growth in the next generation from increased offspring production, while the death rate remains stable. Alternately, the mechanism may be that the limiting agent causes increased immigration and reduced emigration from an area, such that population growth is a result of increased numbers in the current generation, resulting in larger future generations as well. In systems with either of these mechanisms, the limiting agent may be nutrients, water, space, shelter from weather or predators, or oviposition sites. In anurans, reproductive limitation—such as by oviposition sites—has only been demonstrated experimentally in two species (*Eleutherodactylus coqui*: Stewart & Pough, 1983; and *Dendrobates pumilio*: Donnelly, 1989).

There are three ecological positions regarding the number of limiting resources that an organism or population may experience at one time: one, several, or all resources used by that organism. The "law of the minimum" suggests that growth is limited by a single resource at any one time: a plant will grow in response to the addition of its single limiting resource, until it becomes limited by another resource (Von Liebig, 1855). In contrast, Hutchinson (1941) argued that many factors may contribute simultaneously to an organism's success. Taking Hutchinson's argument further, optimality theory suggests that organisms should minimize effort spent seeking non-limiting resources, and maximize time spent searching for and acquiring a limiting resource. One conclusion of optimality theory is therefore that morphology, physiology and behavior will be altered over evolutionary time such that no resource is taken up in excess, and all resources will ultimately simultaneously limit growth of an individual or population (Chapin *et al.*, 1987; Gleeson & Tilman, 1992).

While there is little empirical evidence for organisms adjusting allocation such that all resources equally limit growth, the conditions that prescribe whether organisms will be subject to single versus multiple limitation are of considerable interest. Multiple limitation is predicted by economic models which presume that resources are not equally costly to obtain. Resources have both an absolute cost and costs relative to other resources, known as their exchange ratios (Bloom *et al.*, 1985). The extent to which a resource is limiting will depend on its exchange ratio with other resources, which depends on supply, demand, and the type of reserve being expended to acquire resource (Bloom *et al.*, 1985). Organisms that can be selected to equalize exchange ratios are predicted, as a result, to have multiple limiting factors. Examples of multiple limitation include several plant species in which multiple resources limit productivity (*e.g.* Bloom *et al.*, 1985; Campbell & Halama, 1993; Meekins & McCarthy, 2000). Additionally, in some arthropod, bird, and mammal species, multiple limitation or habitat quality mediation of limiting factors has been observed (*e.g.*, Newton *et al.*, 1994; Joern & Behmer, 1997; Schetter *et al.*, 1998).

Selection cannot also equalize exchange ratios, however, and under these circumstances single limiting resources are predicted. Changing environmental conditions make availability of resources unpredictable, which will restrict the ability of organisms to adjust the allocation of resources such that they limit growth equally. If the exchange ratio of two resources is highly skewed, the organism may experience a situation of diminishing returns, such that increases in effort

yield smaller and smaller increases in availability. At some point, it is not worth the metabolic cost to obtain a new supply of resource, either because it is too difficult to extract from the environment, or because the organism must travel too far to access it. Thus, some systems will retain a single limiting factor. In such cases, organisms are likely to exert intense effort in competition over available resources rather than in seeking new resources (see Discussion).

I studied resource limitation in *Mantella laevigata*, one of approximately 17 species of Madagascan poison frog (Mantellidae). Adults of this species prey opportunistically on mites, ants, and other small terrestrial and flying insects. Males call regularly from defended territories, which include oviposition sites (water-filled phytotelmata: wells in bamboo or tree holes). Females only approach males issuing advertisement calls; after attracting a female, the male leads her to possible oviposition sites (Heying, 2001). Females assess oviposition sites both before and during courtship, and most often abandon courtships only after investigating the oviposition site that the male has led her to. Males engage in one of three territorial strategies, the most dominant and successful of which is to defend their territories against other males, who often attempt to sneak matings in oviposition sites not their own. Males often abandon courtships in order to engage in territorial defense (Heying, 2001). Females do not base mate selection on the quality or length of male calls; choice is based solely on the quality of oviposition sites in a male's territory (Heying, *in prep*). Clutch size is usually one, and females provide facultative maternal care to their tadpoles by feeding them unfertilized trophic eggs (Heying, 2001). Tadpoles obtain most of their nutrition not through maternal provisioning but by cannibalizing fertilized eggs. While mating pairs oviposit on the side of the well—above the water line and out of reach of tadpoles—fluctuating water levels and the lunging of tadpoles often bring fertilized eggs into reach, allowing tadpoles to parasitize the reproductive efforts of mating pairs (Heying, 2001).

The observations that females leave males after assessing oviposition sites, that males leave courtships to defend their oviposition site-containing territories, and that females choose mates based solely on oviposition site quality, all suggest that high-quality oviposition sites may be limiting for *Mantella laevigata*. This hypothesis prompted the current research, the goal of which was to answer the following questions: Does the abundance of wells affect population density in *M. laevigata*, and do wells that are filled with water attract significantly more *M. laevigata* than wells that are not filled?

Organisms with multiple limiting resources are not expected to respond to experiments in which single resources are increased in the short term. The experiment described here, in which oviposition sites were added to a population of *Mantella laevigata*, is therefore a test both of whether oviposition sites are limiting for *M. laevigata*, and of whether oviposition sites are the *only* limiting factor for *M. laevigata*, as it is impossible

to demonstrate the first, without also demonstrating the latter.

MATERIALS AND METHODS

In each of two years (1997 and 1999), I conducted an experiment to test whether oviposition sites ("wells") were limiting for *Mantella laevigata*, by adding artificial wells to plots which were monitored by visual survey before and after addition of those wells. This experimental design followed from Donnelly's (1989) work on the dart-poison frog *Dendrobates pumilio*, which also use wells (bromeliads) as discrete reproductive resources.

Research was conducted on the 510 hectare island of Nosy Mangabe, which lies at the Northern end of the Bay of Antongil, five km south of the town of Maroantsetra, Toamasina province, in northeastern Madagascar (15° 30' S, 49° 46' E). Nosy Mangabe is a "Special Reserve" in the Masoala National Park, consisting mainly of 100 – 400 year old second-growth forest.

From surveys of the island of Nosy Mangabe, six areas were found (2 in 1997, 4 in 1999) in which *Mantella laevigata* were occasionally seen, very few natural treeholes were found, and there were no bamboo stands within 400 m (Fig. 1). The highest density of *M. laevigata* is found in and around bamboo stands, so areas near bamboo stands were avoided to prevent

□ Bamboo stand (not to scale)
▨ Plot Area (not to scale)
● Main Camp
◉ Fisherman's camp
╲ trail
⌐ coastline
† cemetery

Total Area: 510 hectares
Approximately 2.5 km across (E-W)
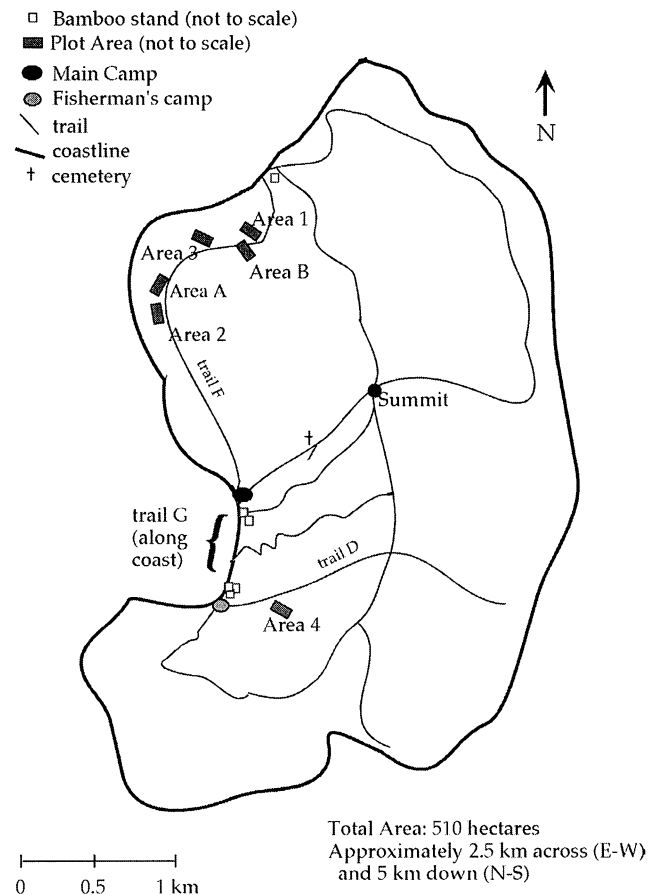and 5 km down (N-S)

0   0.5   1 km

Fig. 1. Plot Areas on Nosy Mangabe.

short-term movement of individuals from known population sources. Individuals were marked by toe-clipping, waistbands, and dorsal tattoos for observation of focal populations in bamboo stands; marked individuals were never observed to move more than 100 m (Heying, unpublished data).

For each of these six unique areas, random compass directions were selected, and three plots were established, in one large transect, with the starting corner 2 meters off the nearest trail, moving in the compass direction chosen. Each transect was 10 m wide and 35 m long, and included three parallel plots which were 10 m long by 5 m wide, each 10 m apart from the next (Fig. 2).

Three days after establishing the plots, visual surveys began. Observers conducted 15 minute visual scans, with established starting points within plots, counting every *Mantella laevigata* adult and juvenile observed. We surveyed the plots approximately every three days. Observers rotated through plots to control for observer effect.

After eight (1997) or six (1999) surveys, during which time each observer had surveyed each plot multiple times, one plot in each area was randomly chosen to receive artificial wells which were filled with water, and maintained ("filled well treatment"). Another plot in each area was randomly chosen to receive wells which were not filled with water, nor

maintained, except to insure that they remained upright and attached to trees ("unfilled well treatment"). The third plot in each area had no wells added to it, and served as a control plot. This design allowed for two comparisons of well use by *Mantella laevigata*: 1) filled *vs.* unfilled *vs.* control plots and 2), by lumping filled and unfilled treatments into a single "well treatment," frog density in control plots could be compared with density in experimentally increased well plots.

Artificial wells consisted of small, brightly colored plastic cups, purchased in the nearby town of Maroantsetra. Seven artificial wells were placed in each treatment plot. In an effort to replicate the naturally occurring variation in well height, diameter, and volume with resources available locally, three sizes of artificial well were placed at each of three heights. Each experimental plot had identical sizes, number, and placement of artificial wells, such that comparisons between plots would be equivalent. In each treatment plot, well 1 had a 100 mm diameter, and a 575 ml capacity (filled wells were maintained at 475 ml); well 2 had a 90 mm diameter, and a 400 ml capacity (filled wells were maintained at 345 ml); and wells 3-7 had a 55 mm diameter, and a 125 ml capacity (filled wells were maintained at 95 ml). Wells 1, 3, and 5 were placed at 0.5 m above the ground; wells 2 and 4 at 1 m above the ground, and wells 6 and 7 at 1.5 m above the ground.

Three days after artificial wells were added to the treatment plots, visual surveys resumed, and continued for 14 (1997) or 18 (1999) more surveys, approximately every three days, as before. I recorded the number of adult and juvenile *Mantella laevigata* seen during each survey; the observer; weather conditions; and time of day (before 1100 h, between 1100 and 1300 h, or after 1300 h).

Experimental set-up and design in 1997 and 1999 were identical, with the following exceptions. In 1997 there were only two experimental areas, in 1999 there were four. In 1997, there were two observers (the author and Jessica Metcalf), in 1999, there were three (the author, Bret Weinstein, and Glenn Fox). In 1997, eight surveys were conducted, over the course of 23 days, before wells were added to treatment plots. Fourteen more surveys were conducted following well addition, over a span of 38 days. In 1999, six surveys were conducted, over the course of 16 days, before wells were added to treatment plots. Sixteen more surveys were conducted following well addition, over a span of 46 days. The experiment was conducted during the rainy season (February through April) in both years.

This experiment was designed to maximally control for as many parameters as possible, while simultaneously allowing for several relevant statistical analyses. Statistics reported in this paper are standard non-parametric tests (Kruskal-Wallis & Mann-Whitney U, analyzed using StatView 5.0.1). Neither of these tests assume that statistically comparable groups have an equal number of data points; thus, differences in number of plots and surveys between years and before and after wells were added to control plots are not problematic for these analyses. In addition, this experimental design controlled for
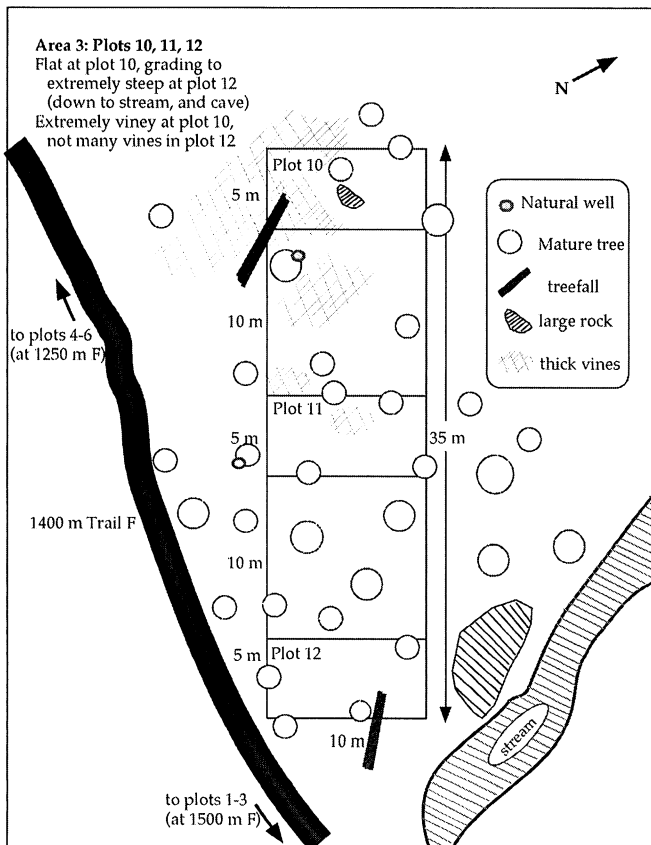

Fig. 2. Physical Environment of Area 3, with parallel plots.

parameters such as effects due to observer, weather, time of day, and differences between years. Differences in frog density between areas was expected, and as all areas were represented in all analyses, merely constitutes noise in the data.

RESULTS

The presence of artificial wells is correlated with an increase in population density in *Mantella laevigata*. The experimental design allows two distinct analyses of these data, both of which find significant differences between plots with wells added to them, and plots without wells added. The first analysis compares population densities on the same plot types (control, unfilled, and filled), before and after wells were added to the treatment plots (see Figure 3). The addition of wells significantly affected population density in *M. laevigata* both in plots to which unfilled wells were added (Mann-Whitney U, U = 1258.0, tied-p = 0.002), and in plots to which filled wells were added (Mann-Whitney U, U = 1473.0, tied-p = 0.040). By contrast, control plots, to which wells were never added, did not differ significantly between the same time periods, although there was an unexpected, non-significant trend for frogs to leave these plots, probably as a result of movement into neighboring treatment plots (Mann-Whitney U, U = 1764.0, p = 0.406, Fig. 3).

The second analysis compares population densities in control (plots to which no wells were ever added) versus treatment (all plots to which wells were added—filled plus unfilled data) plots, after wells were added to the treatment plots (see Fig. 4). When treatments are lumped this way, and treatment plots are compared to control plots for surveys 7 – 24 only (after wells were added), all plots with wells in them attracted significantly more *Mantella laevigata* than did control plots (Mann-Whitney U, U = 5121.5, p < 0.0001, Fig. 4).

Contrary to expectation, unfilled-well plots attracted significantly more frogs than did filled-well plots (Mann-Whitney U, U = 4311.0, p = 0.008), although unfilled-well plots had a non-significantly higher baseline as well (Mann-Whitney U, U = 606.0, p = 0.594, Fig. 5). Due to chance, baseline population densities of control plots were non-

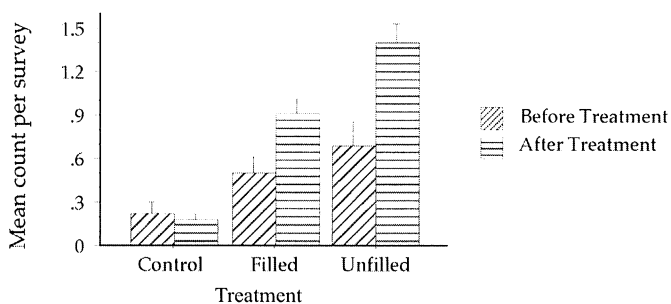Fig. 4. Population density of *Mantella laevigata* is higher in plots with wells added than in control plots. During concurrent surveys, plots with wells added to them attracted significantly more *M. laevigata* than did control plots. Data reported are Means ± S.E. See text for statistical details.

significantly lower than those of either unfilled-well or filled-well plots.

The six distinct areas, in each of which three plots were laid (2 in 1997, 4 in 1999), were significantly different from each other with respect to frog density (Kruskal-Wallis, H = 27.112, p < 0.0001, Fig. 6). Weather also affected the numbers of frogs counted, with cooler, wetter weather generally being correlated with higher numbers of observed frogs (Kruskal-Wallis, H = 11.145, p = 0.0079, Fig. 7). This result is even more clear when the weather data is collapsed into two categories: hot and/or dry, and wet and/or cool (Mann-Whitney U, U = 18608.0, p < 0.0021). Time of day had no significant effect on observed population density (Kruskal-Wallis, H = 2.562, p = 0.2039, Fig. 8), nor did observer (Kruskal-Wallis, H = 2.985, p = 0.2952). Year did have a significant effect on population density of *Mantella laevigata*, with significantly more individuals observed per survey in 1997 than in 1999 (Mann-Whitney U, U = 15893.5, p = 0.0027).

DISCUSSION

Wells are limiting for *Mantella laevigata*. As only two previous studies have experimentally demonstrated population

Fig. 3. Population density of *Mantella laevigata* increases with the addition of wells to experimental plots. Population density increased significantly in "unfilled" plots after the addition of wells, but not in "filled" or "control" plots. Data reported are Means ± S.E. See text for statistical details.
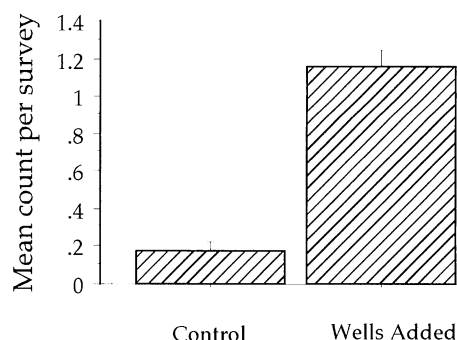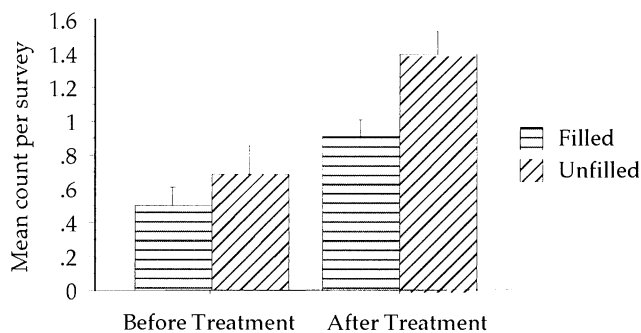

Fig. 5. Effect of unfilled versus filled wells on population density of *Mantella laevigata*. Plots containing unfilled wells attracted significantly more frogs than did plots containing filled wells. Data reported are Means ± S.E. See text for statistical details.
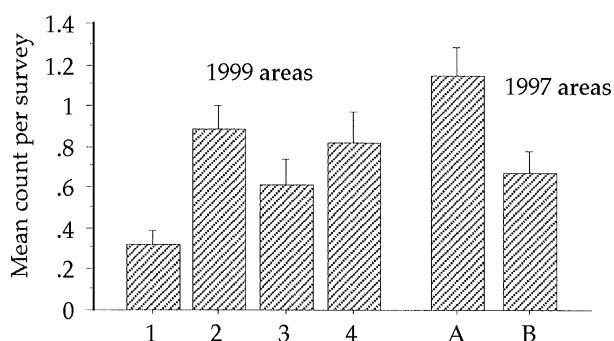
Fig. 6. Area Has an Effect on Population Density of *Mantella laevigata*. Data reported are Means ± S.E. See text for statistical details.
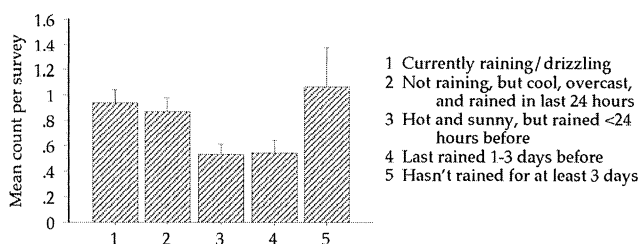
Fig. 7. Weather has an effect on observed population density of *Mantella laevigata*. Data reported are Means ± S.E. See text for statistical details.
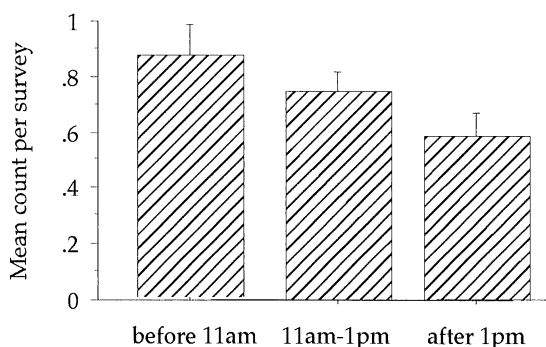
1 Currently raining/drizzling
2 Not raining, but cool, overcast, and rained in last 24 hours
3 Hot and sunny, but rained <24 hours before
4 Last rained 1-3 days before
5 Hasn't rained for at least 3 days

Fig. 8. No time of day effect on observed population density of *Mantella laevigata*. Data reported are Means ± S.E. See text for statistical details.

limitation by reproductive resources in anurans (Stewart & Pough, 1983; Donnelly, 1989), this work provides an important example of what is likely to be a larger trend. My two experimental treatments (unfilled-wells added versus filled-wells added) did not differ, probably because, during the rainy season, unfilled wells quickly fill and remain filled naturally with rainwater. Having a well that is maintained at a constant level and cleaned of excess detritus is an evolutionary novelty, and one that *M. laevigata* should not be expected to respond to if it provides no benefit. Indeed, the addition of rainwater and regular disturbance by humans might be a deterrent, making

the filled wells less desirable than the unfilled ones in times of water excess, although all wells, of both treatment types, were visited and manually disturbed after each survey to control for experimenter influence. During a time of drought, when the unfilled wells were in fact empty of water some of the time, I would predict a difference between these two treatments. During the dry season this might also be true, although much lower rates of breeding during the dry season would make it difficult to collect enough data to test this prediction (Heying, *personal observation*).

Other changes in population density found during this study include that the "control" plots showed a trend towards decreases in population density after the wells were added in the treatment plots. This is likely due to emigration out of those plots, which suggests that the mechanism of limitation in this system is to reduce immigration when resources (wells) are in short supply. Due to the relatively short time period of the study in each year, it is unlikely that observed population density increases were due to an increase in birth rates, although future studies might benefit from describing the age structure of the population before and during the experiment. The populations affected by this study are spatially restricted enough that a local bounty—such as the addition of 14 artificial wells within a single area—apparently causes emigration from the one plot without additional wells.

I failed to falsify my hypothesis that wells are limiting for *Mantella laevigata*. This begs the question, in light of earlier arguments regarding multiple limiting resources: What about this system has caused natural selection to allow a single limiting factor?

Multiple limitation occurs when effort devoted to acquiring a non-limited resource is shunted towards effort to acquire a limited resource. When extra, limited resource can be obtained by exchanging effort in this way, natural selection will tend to equalize availability of multiple resources. For instance, if there were an infinite number of oviposition sites (wells) in the environment of *Mantella laevigata*, the frogs would need only find those wells in order to utilize them. By converting the non-limiting resource of food into the energy used to discover additional (limiting) wells, the frogs could, theoretically, ultimately exist in a system of multiple limitation. But wells are not infinite, and all wells in the unmanipulated system are already being used. No matter how much food energy is put into searching for new oviposition sites, there will be no more; other resources cannot be converted into wells.

Why breed in treeholes? Anuran treehole (well) breeders utilize a distinct niche during their larval stage. In the absence of direct development or extreme behavioral adjustment on the part of the parents (found in the gastric brooding frog, or when one of the parents carries the clutch on its back), which is known from very few anurans, these species must go through both an egg and tadpole stage in which they rely on a single environment. The larval environment is the result of oviposition site choice by one or both parents.

Mantella laevigata occurs on Nosy Mangabe and the Masoala peninsula, in neither of which do small ponds of surface water exist. The fresh water in these forests is found in rivers; small, fast moving streams that flood regularly during the rainy season; and treeholes. In a species that currently uses treeholes, the adaptive valley between this state and the nearest possible one, that of using small, fast moving, often-flooded streams, is vast and deep. Moving between these two adaptive peaks would require a set of modifications in the offspring that would be distinctly suboptimal in these two, divergent habitats (Wright, 1932).

Mantella laevigata has a clutch size of one, which is apparently adaptive in a treehole breeding environment in which parents can keep track of their offspring (Heying, 2001). However, this minimal clutch size would almost certainly condemn a high proportion of eggs laid to either physical mutilation in the fast moving environment of a stream or river, or to predation, especially by fish or dragonfly larvae, known predators of amphibian eggs and larvae in other systems (*e.g.*, Resetarits & Wilbur, 1989; Kats & Sih, 1992; Hopey & Petranka, 1994; Holomuzki, 1995).

Minimal clutch size is possible in *Mantella laevigata*, in part, because of the reduced number of predators in treeholes. Release from predation is a clear advantage to breeding in restricted water bodies. Furthermore, treeholes provide an easily defensible territory for males, which allows them to increase their certainty of paternity by excluding other males. Continuing defense of and courtship in wells already containing tadpoles constitutes, at the very least, passive paternal investment by the male territory holder. Males dissuade other anuran species that breed in treeholes from displacing tadpoles. And eggs resulting from later courtships between the father/territory holder and an unrelated female often go to feed an existing tadpole (Heying 2001). Females gain whenever males invest in offspring. Defense of retreat sites offers advantages to both sexes in *M. laevigata*, which is similar to the system described for *Eleutherodactylus coquí* in Puerto Rico (Stewart and Rand, 1991).

Why be choosy about oviposition site quality? Resetarits (1996) argues that oviposition site choice must be under equally strong selection as egg and clutch size in order to generate locally adapted life history phenotypes and optimize parental fitness. If oviposition site quality is variable, and correlated with offspring success, both sexes are expected to be selective. In *Mantella laevigata*, females are choosing oviposition site and *not* male quality or current condition (Heying, *in prep*), suggesting a particularly important role for oviposition site quality in reproductive success in this species.

Oviposition sites act as patches, each of which contain both resources and risk (Resetarits, 1996). In the Masoala, the resources available to *Mantella laevigata* in high-quality treeholes include territorial defense by the resident male against intruding competitors; and deep and therefore reliable water, which is likely to attract future courtships, thus providing food

for tadpoles in the form of fertilized eggs (Heying, 2001). The risks of low-quality treeholes include desiccation, predation, cannibalism, and competition from heterospecifics.

Desiccation of eggs or larvae is a risk for amphibians. Semlitsch and Gibbons (1990) found that pond drying is inversely correlated with larval success in salamanders. In treehole breeders, the amount of water in the well and the humidity are both known to affect survivorship (damselflies – Fincke, 1994; rhacophorid frogs – Kam *et al.*, 1998). Several studies have revealed a preference for moist or wet oviposition sites in amphibians (treefrogs – Crump, 1991; salamanders – Figiel & Semlitsch, 1995; ranid frogs – Spieler & Linsenmair, 1997). *Mantella laevigata* do not oviposit in dry or shallow wells (Heying, *in prep*).

Predation is reduced in treeholes, but is a prominent source of mortality for most anuran eggs and tadpoles. In several species, adult anurans choose oviposition sites that reduce the risk of predation, and there is evidence from Amazonian frogs that treehole breeding may have evolved as a response to predation pressure from aquatic predators such as tadpoles and beetle larvae (Magnusson & Hero, 1991). Documented pairs of anuran prey with their avoided predators include bullfrogs and leeches (Howard, 1978); treefrogs and both salamanders and fish (Resetarits & Wilbur, 1989); wood frogs and fish (Hopey & Petranka, 1994); squirrel treefrogs and banded sunfish (Binckley & Resetarits, 2002); American toad larvae predated by wood frog tadpoles (Petranka *et al.*, 1994); pickerel frogs and American toads predated by fish (Holomuzki, 1995); and red-eyed tree frogs predated by shrimp (Warkentin, 1999). In treeholes, the risk of predation from salamanders, fish and shrimp is essentially zero, but larval insects can co-occur with tadpoles in treeholes. In Brazil nut fruit capsules, which are analogous to treeholes by virtue of acting as restricted, temporary oviposition sites, poison frog tadpoles are susceptible to predation by both mosquito and damselfly larvae (Caldwell, 1993). In bamboo wells, chironimid and tipulid larvae prey on the eggs of well-breeding rhacophorids (Kam *et al.*, 1998). And crane-fly larvae (*Limonia renaudi* Alexander, Tipulidae) prey on the eggs of at least three anuran well-breeders in the Masoala, including *Mantella laevigata* (Heying, *personal observation*). Adult female *M. laevigata* reject oviposition sites that contain predatory crane-fly larvae (Heying, 2001).

Cannibalism is also a threat to anuran larvae, and is therefore a parameter that females choosing oviposition sites should take into account. Crump (1991) demonstrated experimentally that female treefrogs prefer to oviposit in artificial pools lacking conspecific tadpoles, which are known to be cannibalistic. Similarly, African ranid frogs avoid ovipositing in pools where there are cannibalistic conspecifics (Spieler & Linsenmair, 1997), as do some dendrobatids (Summers, 1999) and leptodactylids (Halloy & Fiaño, 2000). In *Mantella laevigata*, females reject oviposition sites that already contain conspecific tadpoles, though they do not reject sites with conspecific eggs (Heying, 2001).

Competition from other species is well studied in anuran larvae (see Alford, 1999 for review), but evidence of oviposition site choice based on risk of competition is less well documented. In other taxa, male gobies experience competition for nest sites from invertebrates and larger fish (Breitburg, 1987; Kroon *et al.*, 2000), and female salmon compete with related species for breeding space (Essington *et al.*, 2000). Damselflies compete for treeholes with other species of odonates (Fincke, 1992). Adults of one species of treefrog avoid ovipositing in ponds that already contain the competitive larvae of another treefrog (Resetarits & Wilbur, 1989). And in *Mantella laevigata*, it is the males, rather than the females, that discriminate against oviposition sites containing competing species of microhylid frogs and their clutches (Heying, 2001).

Given the risk from desiccation, predation, cannibalism, and competition for anuran larvae generally, oviposition site choice must be critical to reproductive success. In other taxa, oviposition site preference by females has been correlated with increased parental reproductive success. Choosy damselflies show increased offspring survivorship (Siva-Jothy *et al.*, 1995), and choosy pied flycatchers gain an increase in clutch size (Siikamäki, 1995). In *Mantella laevigata*, where oviposition sites are limiting, and both sexes discriminate among these oviposition sites, it is likely that parental reproductive success is enhanced by this parental behavior.

For the same reasons that many resources or factors may be simultaneously limiting, females may select multiple aspects of males/resources when choosing mates. That which is most variable in the population at the time of choice is likely to be that which is most actively chosen by females (as in sparrows, Reid & Weatherhead, 1990). If natural selection is continually readjusting the need for and availability of limiting factors, then we should expect populations to evolve in response to those changes as well. Females will choose mates differently if food is limiting and males control access to the food, than if food is widely available, and males control no resources that females require. Thus, it is important to conduct choice experiments at the same time (during the same season) as limitation experiments, in order to attempt to "match up" what is limiting with what females are choosing.

Conclusions. In any population, some parameter or parameters are limiting population growth. Given the propensity for natural selection to act on parameters that limit survivorship, growth, and reproduction, individuals are expected to be limited by multiple factors under must conditions. In some systems, however, multiple limitation does not occur. *Mantella laevigata* is an example of one such system. The single limiting factor for *M. laevigata* is treehole oviposition sites, a resource so unique in the environment of *M. laevigata* that abandoning treeholes as oviposition sites would result in reproductive failure. Given the current reliance on treeholes for reproduction, *M. laevigata* is expected to shunt effort spent acquiring other resources to finding and/or acquiring oviposition sites. Given a finite number of oviposition sites, however, additional effort acquired through food energy will, ultimately, fail to result in the discovery of additional oviposition sites. If there is still excess food in the environment, which is likely for the opportunistically foraging *M. laevigata*, natural selection might convert those resources into increased competition for oviposition sites. Although competition for oviposition sites is not a resource, per se, but a behavior that can lead to the acquisition of limiting resources, this prediction points to a possible role for non-limiting factors. Non-limiting food could, with increased uptake, result in increased levels of competition for oviposition sites, altering the nature of territorial disputes and, ultimately, the social system.

ACKNOWLEDGMENTS

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LIFE HISTORY OF A WEST AFRICAN TREE-HOLE BREEDING FROG, *PHRYNOBATRACHUS GUINEENSIS,* GUIBÉ & LAMOTTE, 1961 (AMPHIBIA: ANURA: PETROPEDETIDAE)

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ABSTRACT

We report on the life history of a West African tree-hole breeding frog, *Phrynobatrachus guineensis.* This article is based on field data gathered through the years 1999 to 2002 in Taï National Park, Ivory Coast. From May 2000 to September 2002 we monitored breeding sites on a nearly daily basis. In total we have 49,818 data sets covering 89 natural and 57 artificial breeding sites. *P. guineensis* occurs in primary rainforest and reproduces in water-filled tree-holes, fruit capsules and snail shells. Density of potentially suitable tree-holes ranged between 0 and 167 tree-holes per hectare in different parts of the forest. Breeding sites showed a clumped distribution and were situated exclusively in drier parts of the forest. Highest density of breeding sites used through a particular period was 23 per hectare. Water volume of oviposition sites ranged from 4 to 900 ml. Based on mark-recapture data, we calculated a mean population size of 49 reproducing males in an area of approximately five hectares. Breeding activity was highest during the rainy seasons and close to zero during drier parts of the year. Adult males had a total reproductive period of about one month. The longest interval from first to last capture was 193 days for a male, and 130 days for a female. Only 2.3 % of known frogs survived the long dry season of about three months. Most males stayed only for a single day at a particular breeding site. The longest period that a male continuously remained at a breeding site was 23 days. We observed turnover rates of up to 75 % in use of breeding sites between successive rainy seasons. Most often, only single males occupied a breeding site. However, occasionally up to six males shared the same hole. Territorial behavior was rarely observed among males. Adult frogs did not provide parental care. Mean clutch size was 18.7 eggs. Tadpoles hatched after five days, and larval development ranged from 15 to 28 days (mean 20.6 days). Mean tadpole density was 0.6 tadpoles/ml; the highest density was two tadpoles/ml. Mean tadpole mortality was 44.7 %. Predation and desiccation accounted each for about one quarter of the mortality, the remainder was for unknown reasons. The presence of aquatic predators prevent *P. guineensis* from using larger tree-holes that are less prone to desiccation. When fly and dragonfly larvae were present, tadpole mortality raised to 75-100 % per tree-hole.

Key words: Amphibia, Anura, Petropedetidae, *Phrynobatrachus guineensis,* behavior, breeding site characteristics, phytotelmata, population size, ecology, larval development, mortality, reproduction, rainforest

INTRODUCTION

Phrynobatrachus is one of the largest genera of African anurans. Currently, approximately 67 species, occurring exclusively in Sub-Saharan Africa, are recognized (Poynton, 1999; Rödel & Ernst, 2002b). Often several *Phrynobatrachus* species occur in syntopy. In Taï National Park, Ivory Coast, we recorded 13 *Phrynobatrachus* species (Rödel, 2000b). Most *Phrynobatrachus* species deposit 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). Their tadpoles are exotrophic, lentic and benthic (Lamotte & Dzieduszycka, 1958; Altig & McDiarmid, 1999; Rödel, 2000a; de Sá & Channing, 2003). The four known exceptions that deposit eggs terrestrially are *P. alticola,* which oviposits on leaves on the forest floor and exhibits direct development (Rödel & Ernst, 2002a), *P. phyllophilus* which deposits its eggs on leaves close to extremely small puddles on the forest floor (Rödel & Ernst, 2002b), *P. dendrobates* depositing its eggs on humid moss, with eggs guarded by the male (Drewes, 1999), and *P. guineensis.* *P. guineensis* breeds in tree-holes, water-filled fruit capsules, and empty snail-shells of the genus *Achatina* (Rödel, 1998a).

Only a few African anurans are known to breed in

phytotelmata. Perret (1961, 1966) reported *Hyperolius mosaicus* and *H. acutirostris* depositing eggs in tree-holes, and described the biology of the tree-hole dwelling *Acanthixalus spinosus.* Very recently a second species of that genus, *Acanthixalus sonjae,* also breeding in tree-holes, was described from West Africa (Rödel *et al.,* 2003). *Callixalus pictus,* another hyperoliid species, is known to live in broken stalks of bamboo (Schiøtz, 1999). Other African frogs, breeding in tree-holes are bufonids (*Nectophryne afra*: Scheel, 1970; *Mertensophryne micranotis*: Grandison, 1980; Grandison & Ashe, 1983) and microhylids (*Hoplophryne* spp: Noble, 1929). *Phrynobatrachus guineensis* is the only known African petropedetine frog breeding in tree-holes.

Guibé & Lamotte (1961) described *Phrynobatrachus guineensis* from Mont Tonkoui, Ivory Coast. It has been recorded from Guinea and Sierra Leone to western Ivory Coast (Guibé & Lamotte, 1961, 1963; Schiøtz, 1964a, 1967; Lamotte, 1966, 1971; Böhme, 1994; Rödel, 1998a; Frost, 2002; Rödel & Branch, 2002; Rödel & Ernst, 2002b). A general description of *P. guineensis* (Fig. 1; male mean SVL: 16.6 mm, female mean SVL: 18.4 mm) is given by Guibé & Lamotte (1961, 1963), Rödel (1998a) and Rödel & Ernst (2002b). The latter authors showed that older records of *P. guineensis* included *P. phyllophilus,* which is restricted to wetter parts of the forest and reproduces in small puddles on the forest floor (Rödel &
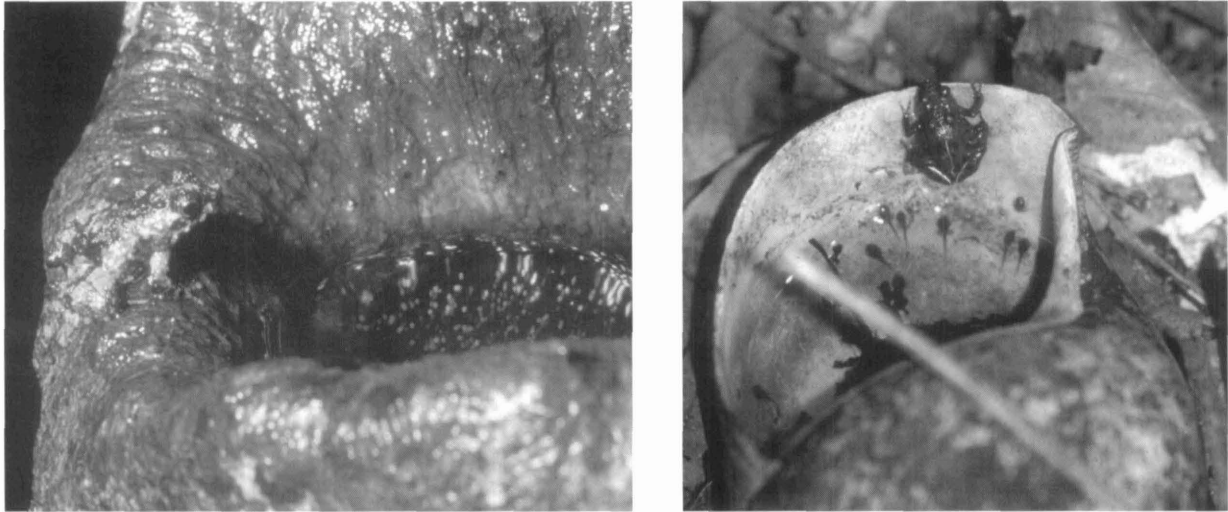
Fig. 1. A: A well camouflaged *Phrynobatrachus guineensis* male at its breeding site, a water filled tree-hole; B: *P. guineensis* male and tadpoles in an empty *Achatina* shell.

Ernst, 2002b). *P. guineensis* attaches small clutches of large macrolecithal eggs (diameter 3 mm) singly above the water surface.

The tadpole morphology is of a general ranoid type. They have a spatulate tail fin that is barely curved and robust jaw sheaths that allow for macrophagous feeding. Frequent surfacing indicates aerial breathing (Rödel, 1998a). This morphology and behavior are common in phytotelmata breeders (Wassersug *et al.*, 1981; Lannoo *et al.*, 1987; Altig & Johnston, 1989). Two *Phrynobatrachus guineensis* call types have been described by Rödel & Ernst (2002b). The *P. guineensis* advertisement call described by Schiøtz (1964b) correctly belongs to *P. phyllophilus* (Rödel & Ernst, 2002b). Apart from these data, nothing is known about the ecology of *P. guineensis*. We herein give a summary of life history data of *P. guineensis*, based on field observations in Taï National Park.

MATERIALS AND METHODS

Study site. With an area of 4,550 km², Taï National Park (TNP) is West Africa's largest protected area of primary rainforest (Guillaumet 1967; Sangaré, 1995; PACPNT, 2000). Situated in south-western Ivory Coast, TNP extends between 5°08'-6°07'37" N and 6°47'-7°25' W, in close vicinity to the Liberian border. Our investigations within TNP were based at the Station de Recherche en Ecologie Tropical (SRET, 05°50.003' N, 007°20.536'W) maintained by the University of Abobo-Adjamé, Abidjan. The station is in the western part of the park approximately 23 km east of the town of Taï.

TNP is situated within the equatorial climate zone and influenced by the southern Passat. The climate can generally be described as humid-tropic seasonal (Riezebos *et al.*, 1994; Parren & de Graaf, 1995; Richards, 1996). Mean annual precipitation reaches 2,100 mm in the southwest and 1,700 mm

in the northeast of TNP with most rainfall occurring during two distinct wet periods (Fig. 2). Daily temperature varies between 20-33°C, the mean annual temperature being 26°C (Rompaey, 1993). Humidity fluctuates between 85 % (day) and 90-100 % (night).

Floristically, TNP belongs to the Guinean-Congo-Region (Guillaumet, 1967; Lawson, 1986; PACPNT, 2000). The potential natural vegetation is evergreen seasonal rainforest (Riezebos *et al.*, 1994; Richards, 1996; PACPNT, 2000). The study site comprised an area of about 30 km² of primary and secondary rain forest in the surroundings of the SRET. The vegetation structure in secondary forest was characterized by the absence of a closed canopy and, consequently, a thick under-storey. In primary forest, the vegetation structure varied
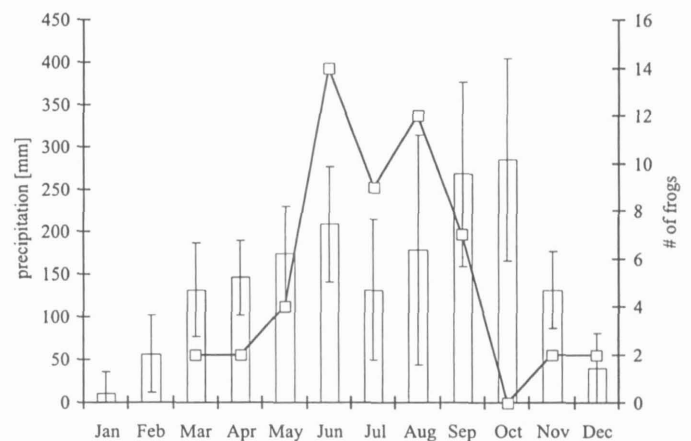

Fig. 2. Monthly precipitation in TNP (1988-2002, bars, given are x ± sd) and *Phrynobatrachus guineensis* specimens (N = 54) recorded on 10 transects in the course of two years (1999 and 2000). Rainfall data for dry season months in 1999, 2000 and 2002 are partly lacking. Mean annual precipitation for the remaining 12 years was 1,806 mm (± 297 mm sd; R. Noë, pers. comm.; I. Herbinger, pers comm. and own data).

from densely vegetated, swampy areas with a low canopy to dry open forest with a canopy exceeding 50 m height in some areas.

Monitoring of Phrynobatrachus guineensis. In the course of a general amphibian-monitoring program (see www.biozentrum.uni-wuerzburg.de/zoo3/roedel_kl.htm), we established 10 rectangular transects, each 600 m in length. Six transects were situated in primary forest around the SRET station (up to 1.9 km distance), four in secondary forest close to the TNP border (up to 4.8 km distance).

Habitats were characterized using several variables, like vegetation cover in four strata, soil type and humidity, and leaf litter density. In order to quantify the availability of all potential breeding sites, every aquatic habitat located at a distance of up to 25 m from either side of the transect was registered. Transects were intensively monitored (0.30-0.35 m/s walking speed), to record all individuals within 100 cm on either side. One transect walk lasted about half an hour. Additionally, all individuals calling within 12.5 m on either side of the transect were counted. For a more detailed description of our transect design and the monitoring scheme see Rödel et al. (2001).

When a calling male of *Phrynobatrachus guineensis* was detected, we tried to localize the potential breeding site by following the calls. We define breeding site here as all sites where a male was found calling at least once, whether or not spawning occurred. Every breeding site was assigned an identification number and then added to a daily *P. guineensis* monitoring program.

We mainly focused on two locations with higher densities of *Phrynobatrachus guineensis* breeding sites. The first area comprised the surroundings of an old logging road, south of the SRET, approximately 2.5 km in length. The second area was situated approximately 500 m to the west of the southern end of that road, and covered an area of about 2 ha of relatively dry primary rain forest. This area covered the middle and northern parts of our transect III. Within this area water filled tree-holes, fruit capsules and empty snail shells were the only open water bodies available.

At the end of 2000, 54 breeding sites had been identified. By September 2002, 89 natural breeding sites were part of the monitoring program. We also established 57 artificial breeding sites that formed part of the daily monitoring program. Each of the latter sites consisted of a water filled snail shell with a Plexiglas window for observation. Breeding sites were only excluded from the monitoring program if they were no longer suitable for breeding (*e.g.*, water loss due to rotting wood).

In the *Phrynobatrachus guineensis* monitoring program, breeding activity, reproduction and larval development was recorded. The monitoring took place on nearly a daily basis from May 2000 to September 2002. In total, we gathered 49,818 data sets, each containing one day's data for each breeding site. Before examining a breeding site in detail, we acoustically monitored five minutes for calling males, at a distance of 5-10 m from the site, to estimate how many males were present before approaching. In nearly all cases we could approach breeding sites without causing the frogs to flee. Adults were captured, measured and sexed (see Rödel & Ernst, 2002b), and unknown adults of both sexes were marked individually by toe clipping the hind feet (*e.g.*, Heyer *et al.* 1994). Frogs were released at the site of original capture.

Many holes were difficult to monitor, because the shape of some holes prevented accurate counts of eggs, tadpoles, or even present males. We recorded the daily presence and number of adults, clutches, eggs, tadpoles and juveniles. To facilitate this census, eggs and tadpoles were assigned to stage classes (Table 1). The water level was measured daily to estimate the water holding capacity of each site. Water level was measured at the deepest point of the tree-hole and included the substrate accumulated on the bottom plus the water column. Dried up tree-holes were recorded as zero values.

To characterize breeding sites we took several measurements at each site. Most measurements were taken once at the beginning of every field season. Tree holes were categorized as: 1. holes in a living tree trunk, 2. holes in a root, 3. holes in a rotten tree, 4. fruit capsules and 5. snail-shells. For every hole we determined the entrance area (accuracy: ± 0.1 cm^2) and the maximal possible water surface (± 0.1 cm^2). Sediment depth was measured with a stick and a ruler at the deepest point (± 0.1 cm). Maximum possible water depth (± 0.1 cm) was defined as the point where water could spill out of the tree-hole. To measure the water volume (± 1 ml), the water was carefully sucked out with a flexible tube and a 50 ml syringe until no

Table 1. Stage classes assigned to eggs and tadpoles in the monitoring program. Stage classes of eggs represent days after oviposition. Tadpoles according to Gosner (1960).

Stage Class	Definition	Gosner Stage
1	Jelly not swollen (1st day)	1-8
2	Jelly swollen, embryo round (2nd day)	9-17
3	Embryo enlarged, not moving (3rd day)	18-21
4	Embryo moving (4th-5th day)	22-24
1	Hatched larvae, rarely free swimming (first three to five days after hatching)	25-30
2	No visible hind legs	31-35
3	Hind legs visibly emerged	36-41
4	Hind and fore legs visibly emerged	42-43
5	Tail at least partly absorbed	43-46

water remained above the substrate. After that treatment, the water was returned into the hole. The pH (± 0.01) and the oxygen concentration (± 0.01 mg/l) were measured on 24–27 August 2001 with a multi parameter water tester (WTW© Multiline 3F: with pH/oxi 340i electrode). Measurements were taken on three consecutive days, and mean values calculated. Nitrite was measured on 1–2 November 2001 with a chemical colorimeter test (Aquamerk© Nitrite Test, ± 0.01 mg/l) in 38 holes. Tadpoles occurred at eleven of these sites. Nitrite (NO_2) was measured, because it is a product of ammonia which is the general form of nitrogenous waste excreted by tadpoles (Duellman & Trueb, 1986). At 16 tree-holes water and air temperature were recorded with small real time temperature recording devices (iButton-TMEX, version 3.12, ± 0.1 C°) as follows: In four different areas within the monitoring site we randomly chose two oviposition sites and two tree-holes not used for oviposition. At every hole, air and water temperature was recorded every 30 minutes for ten consecutive days. In each tree-hole one button was placed approximately 2-4 cm below the water surface, and one button was attached close to the entrance outside the water. Direct exposure of buttons to sunlight was prevented. We examined tree-hole variables of 87 natural breeding sites. Precipitation data were taken from the Taï Monkey Project (TMP).

From September to October 2001, we also recorded the presence and abundance of the aquatic invertebrate fauna with a body length > 3 mm and of non-aquatic egg predators every seven days for five consecutive weeks in all breeding sites. Identification was to order or family level. Voucher specimens were subsequently sorted to morphospecies. Some larvae were kept and raised to metamorphosis for better identification.

Throughout the years (from February 1999 to September 2002) we adjusted the monitoring program to improve data quality for specific questions. Breeding sites varied in accessibility which restricted data acquisition to a certain degree. Therefore, we restrict analyses in some cases to parts of the data to guarantee comparability (see Results).

RESULTS

Habitat selection in Taï National Park. In 1999 and 2000 we performed 552 transect walks, 120 in 1999 (February 27 - December 20) and 432 in 2000 (March 15 - September 23). In 1999 we recorded a total of eight adult *Phrynobatrachus guineensis* (0.13 frogs/hour). In 2000 we recorded 46 adult specimens (0.21 frogs/hour). Of these, 41 were recorded during 259 transect walks in primary forest, and five were recorded during 173 transect walks in secondary forest. *P. guineensis* was significantly more abundant in primary forest transects (Fishers exact test, two tailed, p < 0.001). All 54 specimens were recorded during daytime. Only seven breeding sites were within the range of our transect recordings. Most *P. guineensis* therefore were recorded far away from breeding sites. Fifty-one specimens were recorded in drier parts of the forest (no

Table 2. Location of 89 natural breeding sites used at least once by male *Phrynobatrachus guineensis*, records from 2000-2002.

Type of breeding site	Number of sites
in a living tree trunk	43
in a root	14
in a rotten tree	24
fruit capsule (*Strychnos* sp,; Loganiaceae)	5
snail shell (*Achatina* sp.)	3

puddles, ponds or creeks), whereas only three specimens were recorded in swampy areas. The vast majority of frogs were recorded during June to September (Fig. 2).

Breeding site characteristics. We recorded the highest density of *Phrynobatrachus guineensis* breeding sites in a relatively dry forest, without open water other than in tree-holes and snail shells. The low under-storey (0-0.5 m height) was absent or only sparsely developed. The shrub-storey (0.5-1.5 m) was present but sparse. The subcanopy tree-storey (3-10 m) was nearly closed and the canopy (> 20 m) was present but not closed. Table 2 summarizes the type of all natural breeding sites in the *P. guineensis* monitoring program.

We randomly checked 11 trees with *Phrynobatrachus guineensis* breeding sites for species identity. The trees belonged to five families, eight genera and nine species: Melastomataceae: *Warneckea guineensis* (Syn: *Memecylon guineensis*), *Memecylon lateriflorum,* Caesalpiniaceae: *Anthonotha macrophylla, A. sassandraensis,* Euphorbiaceae: *Drypetes pelegrini, Maesobotrya barteri* (2 specimens), Ebenaceae: *Diospyros canaliculata* (2 specimens), Icacinaceae: *Leptaulus daphnoides,* Rubiaceae: *Corynanthe paehyceras.*

We examined habitat characteristics of 89 natural breeding sites (Table 3). *Phrynobatrachus guineensis* was never observed in breeding sites with water depth lower than 1 cm and entrance diameter smaller than 1 cm. The entrance of used tree-holes never exceeded 261 cm in height. In tree-holes that were not used for oviposition, the median volume was 39 ml (N = 38), similar to the median volume of tree-holes used for oviposition (36 ml, N = 32). The largest recorded volume in the study area was 1000 ml and the smallest volume 3.7 ml. This is comparable to the largest volume (900 ml) and the smallest volume (3.7 ml) measured in tree-holes used for oviposition. In other parts of the forest we found tree-holes with water volumes of more than a hundred liters. These large tree-holes were never used by *P. guineensis,* but most often contained dragonfly larvae and *Acanthixalus sonjae* tadpoles (Rödel *et al.,* 2003).

No significant differences in air and water temperature were detected between used and unused habitats for the daily mean, maximum or minimum of water or air temperature (t-test, p > 0.05, df = 15; N = 16). Water temperature ranged from 21.8-25.3°C (mean = 23.4°C; sd = 0.9°C). Mean temperature never differed more than 0.6 °C between air and water in a tree-hole. Nitrite concentration varied considerably between breeding sites (Table 3), but did not differ significantly between tree-

Table 3. Variables of 89 natural breeding sites (2000-2002). Not all variables could be measured at all holes. For data sets that differ considerably from normal distribution we give mean and median, and standard deviation and 25/75 quartiles, respectively; N = # of breeding sites (regardless if living tree, root, rotten wood, snail or fruit capsule), * relative water presence = (days with water present / observation days) * 100.

Habitat variables	Mean/ median	sd/25-75% quartiles	Min	Max	N	
Height above ground [cm]	58.1	59.9		2.0	261.0	50
diameter of tree trunk with hole [cm]	17.7	10.9		5.5	42.0	44
entrance area [cm²]	34.2 / 21.0	59.7 / 11.4 - 32.1	1.8	370.5	74	
hole volume [cm³]	181.5	198.3		3.8	824.7	44
water volume [ml]	106.8 / 39.0	189.9 / 19.1 - 120.0		3.7	1000.0	58
max. possible water surface [cm²]	44.8 / 21.0	68.9 / 7.0 - 61.0	2.0	371.0	59	
max. possible water depth [cm]	6.2 / 4.0	5.0 / 3.0 - 8.8		2.0	30.0	64
relative water presence [%]*	84.3 / 93.5	21.3 / 73.4 - 100	37.0	100	37	
sediment depth [cm]	1.8 / 1.0	2.1 / 0.4 - 2.3		0.0	9.5	65
pH	6.4	0.9		4.5	7.9	32
O_2 concentration [mg/l]	2.4	1.0		0.5	4.3	26
nitrite [mg/l]	0.025	0.161		0.00	0.75	38

holes with tadpoles (N = 24) and without tadpoles (N = 14; Mann-Whitney –U-test; p > 0.05; Z = -0.686; df =1, N = 38).

Breeding site density, distribution and availability. In August/September 2000 we determined the exact location of 33 breeding sites within or near transect III. Data analysis was restricted to that area because only there could the distances between a larger number of breeding sites be accurately determined. We found a mean of 8.25 breeding sites/ha. Highest density was 23 breeding sites/ha. Breeding sites showed a significantly clumped distribution (nearest-neighbor, R = 0.72, z < 1.96, p = 0.05; Krebs, 1999).

To assess whether the clumped abundance of *Phrynobatrachus guineensis* in different parts of the forest was due to limitation of potential breeding sites, we recorded the availability of potential breeding sites in August/September 2000. In each of six primary forest transects (TI-TVI), we examined twelve randomly selected 25 m² plots (25 plots in transect TIII) for potential breeding sites. Every plot was thoroughly checked once after heavy rain. We defined all tree-holes as potentially suitable that had an entrance diameter of more than 1 cm and a water depth of more than 2 cm. This matched the characteristics of the smallest known breeding site (see Table 3). Bushes and trees were checked for tree-holes up to a height of 300 cm. All tree-holes were characterized in accordance with the regular monitoring scheme.

Tree-holes that fitted the general requirements of *Phrynobatrachus guineensis* were present in all parts of the forest (Tables 4 and 5). Transect I and IV were swampier than transect III and had densities of tree-holes that nearly matched those of transect III. Transect II was in a forest type comparable to transect III and had an even higher density of potential breeding sites. The characters of the non-used holes were well within the range of known breeding sites (compare Tables 3 and 5). However, breeding P. *guineensis* were only recorded in the area of transect III.

Phrynobatrachus guineensis *activity 2000-2002.* We compared the monthly amount of precipitation, presence of male frogs at breeding sites (N = 735, incl. recaptures), number of used breeding sites (N = 432, incl. repeated countings), calling activity (calling activity prior to search for frogs at breeding sites; N = 2006, see methods) and breeding activity (N = 530, only new clutches) in the course of three years.

Number of male frogs encountered, used breeding sites, calling activity and spawning events were always higher during rainy than during dry seasons. However, only monthly calling activity was significantly correlated with amount of rainfall (Table 6).

We additionally analysed spawning events from October 2001 to September 2002 on a daily basis. The number of monitored breeding sites was always the same (N = 119) during that period and consisted both of natural and experimental sites. During that period, 313 clutches were deposited in 39 natural

Table 4. Number of potentially available breeding sites recorded on random plots (25 m²), calculation of breeding sites per ha and number of tree-holes used by *Phrynobatrachus guineensis* (compare text).

Transect	m²	Recorded # tree holes	calculated # / ha	# of used tree holes	N (# 25 m² plots)
I	300	2	66.7	0	12
II	300	5	166.7	0	12
III	625	6	96.0	3	25
IV	300	2	66.7	0	12
V	300	1	33.3	0	12
VI	300	0	0	0	12

Table 5. Summary of the 13 tree holes in transects I-VI (see Table 4) that were not used by *Phrynobatrachus guineensis*.

	height (cm)	max. water depth (cm)	water volume (ml)	sediment depth (cm)	diameter of hole (cm)	tree trunk diameter (cm)
mean	68.2	5.8	41.1	3.6	2.5	7.5
sd	40.7	3.8	42.1	5.1	1.6	5.3
min	12.0	1.5	4.7	0.1	1.0	2.5
max	135.0	16.0	127.3	15.0	6.0	20.0

Table 6. Summary of correlations (Spearman Rank correlation with sequential Bonferroni correction; Rice, 1989) between precipitation (rain), number of male frogs present at tree holes (frog), number of used tree holes (hole), number of calls emitted (calls recorded in five min intervals prior to data collection at each breeding site) and number of clutches (clutch). All data pooled on a monthly base; N = # of months.

variable 1	variable 2	N	R	p	corrected α	
clutch	hole	23	0.8851	< 0.0001	0.005	s
clutch	call	23	0.7220	0.0001	0.0056	s
hole	call	23	0.7128	0.0001	0.0063	s
rain	call	23	0.6355	0.0011	0.0071	s
clutch	frog	21	0.6310	0.0022	0.0083	s
hole	frog	21	0.4889	0.0245	0.01	ns
hole	rain	23	0.3922	0.0642	0.0125	ns
frog	call	21	0.3962	0.0754	0.0167	ns
frog	rain	21	0.3541	0.1153	0.025	ns
clutch	rain	23	0.3430	0.1091	0.05	ns

and 14 artificial breeding sites. Monthly clutch numbers and rainfall were significantly correlated (Spearman rank, R = 0.704, p = 0.011, N = 12, Fig. 3). The total amount of rainfall during that period was exceptionally low (1,351.25 mm for a 12 month period).

In August/September 2000 we investigated the diurnal calling activity of *Phrynobatrachus guineensis*. We observed randomly chosen males for 30 min and counted all calls within this time period. Observations took place from 0630-1900 hours, dividing the observation day into 25 sample units of 30 min. Each unit was sampled at least once (1-36 times, see Fig. 4). Calling activity was lowest during midday (1200-1500 hours) and highest during the morning hours. During night we heard calling *P. guineensis* only twice.

Population size and reproductive lifetime of males. Based on mark-recapture data we estimated the size of the male population of *Phrynobatrachus guineensis* in our study site (5 ha). In total 394 males were marked, 87 in 2000, 154 in 2001 and 153 in 2002. We pooled all captures and recaptures of one week (Fig. 5). Analysis was done with the Jolly-Seber procedure (program Ecological Methodology, © C.J. Krebs 2002).

In total, we have data for 57 weeks (Table 7). Mean weekly population size of reproductive males was 48.6 males (± 16.7 sd, range 11-197). The population estimates did not differ significantly between years (44-70 frogs, Kruskal-Wallis-test, $\chi^2 = 3.588$, df = 2, p = 0.175). This was also the case for the minimum population estimates ($\chi^2 = 5.870$, df = 2, p = 0.053) and the maximum estimated survival rates ($\chi^2 = 1.191$, df = 2, p = 0.551). However, the maximum population estimate ($\chi^2 = 8.762$, df = 2, p = 0.013) and the minimum estimated survival rates ($\chi^2 = 15.811$, df = 2, p < 0.001) differed significantly between years. Maximum population estimates were highest and minimum survival rates were lowest in 2001 (Tables 7, 8).

From May 2000 to September 2002, we recorded the time between first and last capture of 394 individual males. The number of males was negatively correlated with time interval

Fig. 3. Correlation of number of clutches (bars) and precipitation (line) in the course of one year (October 2001-September 2002) at 119 breeding sites (91 natural, 28 artificial; Spearman rank, R = 0.704, p = 0.011, N = 12).
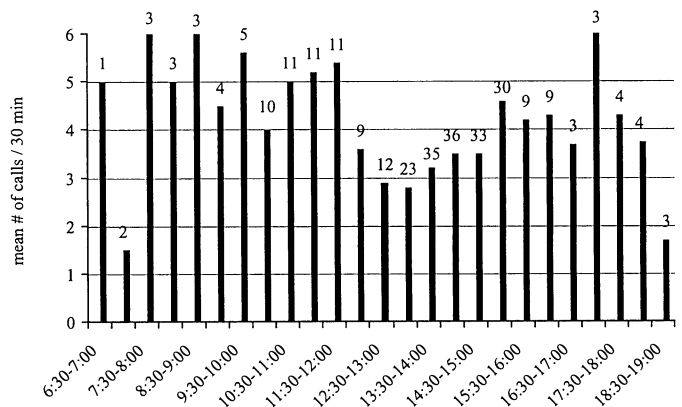

Fig. 4. Calling activity of *P. guineensis* during the rainy season (August/September 2000). The number above the bars refers to the number of 30 min sample units per daytime.
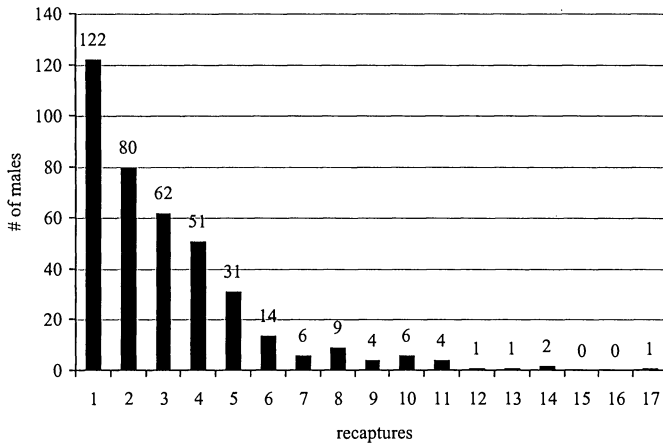
Fig. 5. Weekly recaptures of individually marked *Phrynobatrachus guineensis* males. 122 males have been only captured within one week. One male was recaptured throughout 17 weeks.

Table 8. Minimum and maximum survival rates [%] between weeks, compare Tab. 7. Minimum survival rate was significantly different between 2000 and 2002 (Scheffé-Post-hoc procedure, mean difference [1st-2nd] = -0.2505, p = 0.008) and between 2001 and 2002 (mean difference = -0.2501, p < 0.001).

	minimum survival rate			maximum survival rate		
year	2000	2001	2002	2000	2001	2002
mean	0.47	0.47	0.74	0.95	0.99	0.99
sd	0.15	0.26	0.13	0.10	0.02	0.02
min	0.24	0.07	0.53	0.76	0.91	0.91
max	0.66	0.98	0.96	1	1	1

(Spearman rank correlation, R = -0.731, p < 0.001). 102 males were only recorded once. Mean time from marking to last capture was 35.2 days (± 42.6 days sd). One male was observed 193 days after first capture. Only nine males were recorded in the beginning of the next rainy season (eight males from 2000 to 2001, one from 2001 to 2002). These frogs were observed over a period of 97-174 days (143.3 ± 28.1 days, mean ± sd). This is a conservative estimate as some males arrived at the breeding sites before we started data acquisition in May 2000 and after finishing data collection in September 2002. Individual frogs grew only little during the study period.

Male presence at breeding sites and migration distances. The presence of males at breeding sites was analyzed from June 2001 to September 2002. We analyzed a total of 62 natural and 12 experimental sites that have been used for breeding activities by *Phrynobatrachus guineensis* throughout that time, during which we registered 266 males.

We defined the presence of a certain male at a particular potential breeding site as continuous if we did not fail to detect it for more than two consecutive days. Due to shyness of males and/or tree-hole parameters we could sometimes not catch and identify detected males. In these cases we assigned them to known individuals that we had caught immediately (1-2 days) before and after that event at the same hole. This procedure was restricted to cases where only one male has been monitored during the respective period at the respective breeding site. In some particular cases, color patterns (orange vertebral bands or lateral spots) could be used to make individual identification more secure. We defined visited sites as all sites that had been used by a particular male during the study period. Switches were defined as all changes between breeding sites of a particular male during the study period.

Two thirds of the males (558 males including double counting of marked males) stayed only for one day at a given site. The longest period a male continuously stayed at one site was 23 days. More than 60 % of males visited only one breeding site. However, more than 25 % visited two and the remaining 13.6 % three to five different sites. About 40 % of the males changed breeding sites at least once. One male switched nine times between five different sites (Table 9).

We calculated the distances between two or more different breeding sites that particular males visited (N = 16). Migration distances ranged from 2-94 m (mean = 24 m; sd = 31 m; N = 16). The fastest move was 80.3 m in one day. There was no correlation between migration distance and time between two records.

Breeding site turnover and female presence at breeding sites. We analyzed the turnover of natural breeding sites between consecutive rainy seasons, *i.e.*, the proportion of tree-holes used during one season only, separating first and second rainy season within a year (compare Fig. 2). We separately tested for presence of males and spawning events at a particular site, regardless whether a site was used by only one or by several

Table 7. Summary of population estimates. Given are mean, standard deviation and range for weekly mean, minimum and maximum estimates (only males, Jolly-Seber); maximum population size was significantly different between 2000 and 2001 (Scheffé-Post-hoc procedure, mean difference [1st-2nd] = −138.4378, p = 0.039) and between 2001 and 2002 (mean difference = 162.9814, p < 0.001); N = number of weeks with estimates per year.

per year	2000 values, N = 9			2001 values, N = 20			2002 values, N = 28		
	mean	min	max	mean	min	max	mean	min	max
mean	44.3	30.21	92.0	69.9	37.1	230.0	50.3	42.9	67.5
sd	18.6	13.4	52.1	46.0	17.2	218.0	17.9	15.6	23.2
min	24.9	15.2	39.5	19.3	9.8	26.0	13.1	11.1	17.1
max	84.0	58.9	188.0	197.0	82.8	762.0	84.9	72.3	113.0

Table 9. Number of different breeding sites that a particular male (N = 266) visited and number of switches between breeding sites of these males (June 2001 to September 2002).

frequency	visited breeding sites		changes between breeding sites	
	males	percent	males	percent
0			162	60.9
1	162	60.9	43	16.2
2	68	25.6	29	10.9
3	24	9.0	12	4.5
4	6	2.3	8	3.0
5	6	2.3	5	1.9
6	0	0	2	0.8
7	0	0	3	1.1
8	0	0	1	0.4
9	0	0	1	0.4

males, or contained only one or several clutches, respectively.

The turnover rate in breeding sites used by males was between 37.8 and 63.2 % (Table 10). Turnover rate in using a particular site for spawning was 26.1-75.0 %. A high proportion of tree-holes were used in one season only. Turnover rates between years were higher than between two consecutive rainy seasons within a given year.

We observed a much smaller number of females at breeding sites than males. In 2000 - 2002 we recorded 115 females, and marked 51 of them. In three cases we observed two females at one breeding site. Forty-one females were only captured once, nine were captured twice and one was captured five times. The latter ten females were recaptured at the same or nearby site after one (2 females), three, five, six, 13, 19, 42, 123 and 130 days, respectively. Thirty times we observed a newly deposited clutch at a breeding site where a female had been observed the same day or the day before.

Behavior. Several times we observed males leaving their holes during the day to forage for small arthropods on the forest floor. This foraging period lasted generally only a few hours.

During 3,070 encounters of males at breeding sites, 2,418 times only a single male was observed. In 510 events two males were present, during 98 observations we observed three males, in 35 cases we recorded four males, eight times we observed five males and once we observed six males occupying the same breeding site.

In 2000 we counted calls per male and hour, four times in holes with 2-3 males and four times in holes with only one male, respectively. In these recordings the individual calling

rate was higher in holes with more males (3.6 calls/hour and male) than in breeding sites with one male only (2.4 calls/hour and male). However, sample size was too small to statistically test for differences.

Four times we observed solitary males and amplectant couples together in one hole. In two cases solitary males tried to intercept the couples that consequently left the hole. In one case spawning occurred. The solitary male (male # 2) tried to amplect the couple. Male # 1 uttered release calls during these trials. Male # 2 tried at least five times to pull # 1 off the female. Despite these interruptions the couple continued spawning. After spawning, the female left the hole. The two males remained in the hole and started uttering normal advertisement calls five minutes after the female had left. Ninety percent of the eggs were infertile in this clutch.

On rare occasions, we observed aggressive interactions between males. Once a male approached an already occupied snail shell. While approaching the intruder uttered click sounds. While calling, he alternately raised the right and left arm, then he jumped into the hole. No further interactions could be observed. On several occasions amplectant frogs proved to be two males, sometimes remaining amplectant for more than one hour. The amplecting specimen uttered a very weak version of the advertisement call. In one case a male released the other male after 45 minutes, only to become immediately amplected himself.

Clutch size, embryonic and larval development. For clutch size and phenology we only took clutches into account that allowed estimation of the initial clutch size (data: June 2001 to October 2002, N = 256 clutches, Table 11). Mean clutch size was 18.7 (± 6.8 sd) eggs. Clutches varied in size from two to 35 eggs. Clutches comprising more eggs were deposited by several females. Maximum egg count in one hole was 68. Clutch size differences between months approached significance (Kruskal-Wallis, χ^2 = 12.02, df = 6, p = 0.062).

We analyzed the length of embryonic and larval period (15-19 holes, 25-60 clutches, respectively). Mean duration from spawning to hatching was five days (range: 4-7). Mean larval period was 20.6 days (± 3.1 days sd, range: 15-28). Survival rates in eggs were low (mean = 42.6 % ± 29.7 % sd). Among tadpoles survival rates were lowest in the first stage (mean 73.6 % ± 36.2 % sd). Tadpole survival from the second to the third and from third to fourth stage class exceeded 90 %. Freshly metamorphosed frogs measured about 4 mm.

Table 10. Observed breeding sites, breeding site use and turnover of breeding site use in different rainy seasons for males and spawning events. Only tree-holes that were monitored in two consecutive seasons were included. Turnover of tree-hole use: [100*(no. of tree-holes used in season (x) only + no. of tree-holes used in season (x+1) only)/ total used tree-holes].

season	males				spawning			
	VIII-X (2000)	V-VII (2001)	VIII-X (2001)	V-VII (2002)	VIII-X (2000)	V-VII (2001)	VIII-X (2001)	V-VII (2002)
monitored	60	77	96	96	60	77	96	96
used	52	25	54	36	37	18	42	30
turnover [%]		63.2	37.8	52.5		75.0	26.1	56

Table 11. Monthly variation of clutch size from May to November (data from June 2001 - September 2002). Data for June to September comprise data from both years (N = 256), sd. = standard deviation.

month	clutch #	mean egg # / clutch	sd. egg #	range egg #
May	22	20.7	6.9	6-33
June	24	18.7	5.5	4-35
July	30	15.8	6.4	2-31
August	71	19.6	7.0	5-33
September	66	17.7	7.1	5-35
October	39	20.0	6.9	7-30
November	4	15.8	3.0	12-19

Table 12. Syntopic invertebrate taxa at potential *P. guineensis* breeding sites. FT = feeding type; O = omnivorous; D = detritivorous; P = predator; TP = tadpole predator, EP = egg predator; h = # of tree-holes; F = frequency of the tree-holes in which a taxon was found; FB = frequency of sites that were used at least once by *P. guineensis* for spawning (N = 36); † = without artificial breeding sites; S = snail shell, FC = fruit capsules; m = mean number of individuals per tree hole and DE = mean # of individuals ± sd per 10 ml calculated for the 36 tree-holes used by P. guineensis. N (observed breeding sites) = 78; N (tree-holes) = 19; N (snail shells) = 2; N (fruit capsule) = 3; N (artificial breeding sites) = 54; N (habitats used by *P. guineensis*) = 36, includes artificial breeding sites.

taxa	FT	h # †	F [%]‡	S‡	FC	FB [%]	m	DE
Diptera								
Culicidae sp. 1	O	23	29.5	9	2	45.2	2.6	1.2 ± 1.5
Culicidae sp. 2	O	32	41.0	4	3	35.5	1.5	1.3 ± 1.5
Aëdes sp.	O	16	20.5	0	2	22.6	4.3	1.4 ± 1.8
Thoxorhynchites sp.	TP	26	33.3	0	3	38.7	0.5	0.8 ± 1.1
Tabanidae	O	21	26.9	1	2	35.5	0.6	0.9 ± 1.2
Chironomidae	O	2	2.6	0	3	6.5	0.4	1.1 ± 0
Trichoptera								
species 1	O	3	3.8	0	0	6.5	3.2	1.7 ± 1.9
species 2	O	2	2.6	0	0	3.2	0.5	4.0 ± 4.2
Hymenoptera (Formicidae)								
Crematogaster sp.	EP	5	6.4	2	0	12.9		
Coleoptera								
Helodidae sp.	D	47	60.3	0	1	54.8	1.5	0.3 ± 0.3
Odonata (Libellulidae)								
Hadrothemis camarense	TP	7	9.0	0	0	9.7	0.4	0.1 ± 0
Annelida								
Annelidae	O, D	13	16.7	0	0	12.9	0.7	0.5 ± 0.4
Arachnida								
Arachnidae	P	4	5.1	0	0	3.2	1.0	
Mollusca								
Urocylidae sp.	EP	2	2.6	0	0	6.4		

Tadpole density, tadpole mortality and syntopic tree-hole fauna. From June to September 2001 we recorded tadpole density at 16 breeding sites. The calculation of tadpole density was based on the water volume of a breeding site at day of density estimation. Water volume of the 16 breeding sites varied between 9-278 ml (82.5 ± 88.3 ml, mean ± sd). The mean tadpole density was 0.57 tadpoles/ml (± 0.26; N = 48) and the highest recorded density was two tadpoles/ml.

We recorded a large number of different invertebrates occurring in tree-holes, fruit capsules and snail shells. Most taxa were detritivorous, omnivorous, or filter feeders. Egg predation could only be confirmed for *Crematogaster* ants and slugs (Urocylidae). Dragonfly (*Hadrothemis camarense,* Libellulidae) and fly (*Thoxorhynchites* sp., Culicidae) larvae were observed feeding on tadpoles. Spiders were large enough to be potential predators of both metamorphosing and adult

frogs. Frequency and density of the different invertebrates in habitats used and not used by *Phrynobatrachus guineensis* are summarized in Table 12.

To calculate the egg mortality caused by predation, we compared all fertilized eggs from all clutches with the number of eggs that disappeared before hatching (June - November 2001). During that time the mean predation rate on eggs among all tree-holes was 45.1 % (± 33.7 % sd, N = 48 clutches). In most cases only single eggs disappeared, but in eight cases the whole clutch vanished.

In tree-holes with dragonfly larvae, tadpole mortality was 100 % after one or two days. In habitats with fly larvae mean tadpole mortality was 75.1 % (Table 13). Two out of five tree-holes with dragonfly larvae were used for oviposition during July 2001 - November 2001, but tadpoles were never recorded there. We experimentally introduced 22 tadpoles in

Table 13. Summary of tadpole mortality in 16 breeding sites, monitored from 30 June to 27 November 2001. Given are the mean mortality of clutches that were subject to different predators (dragonfly - *Hadrothemis* - or fly - *Thoxorhynchites* - larvae), drying up of breeding sites or no such event (N = 49). In total 476 tadpoles were monitored, 213 tadpoles died, total mortality therefore was 44.7 %.

habitat	mean mortality [%]	sd. [%]	N = # of clutches
drying out	100	0	9
with dragonfly larvae	100	0	2
with fly larvae	75.1	25.3	6
no drying out or predators	31.5	30.25	32

six tree-holes containing dragonfly larvae. In all holes tadpoles disappeared within one or two days. In another experiment the predators were removed from these six tree-holes, then 47 tadpoles were introduced. A comparison of the mean number of days tadpoles survived with and without predators showed that tadpoles with dragonfly larvae survived significantly shorter than without predators (Fischer Exact test; p = 0.014, $\chi^2 = 6$, df = 1, N = 6). However, larval mortality caused by predators only summed up to 25.5 % of the overall mortality. A nearly equal number of tadpoles died because their holes dried up. The reasons for more than 50 % of tadpole mortality remained unknown (Table 13).

DISCUSSION

Habitat selection. To date *Phrynobatrachus guineensis* has been recorded from primary rainforest exclusively (Schiøtz, 1964a, 1967; Böhme, 1994; Rödel, 1998a). The only known exception, one specimen recorded from a savanna on Mont Nimba (Guibé & Lamotte, 1963), is most likely based on a misidentification. This study confirms the dependence of *P. guineensis* on primary rainforest, in which it is mainly restricted to drier parts. There it reproduces exclusively in small water-filled tree-holes, snail shells and fruit capsules. In tropical forests small pools are generally rare on the forest floor, thus several species of various families use phytotelmata for reproduction (Duellman & Trueb, 1986). These habitats might allow escape from high interspecific competition and from high predation pressure in pools. *P. guineensis* succeeded in colonizing parts of the forest that are not accessible for reproduction to most other frogs in TNP. The only other frogs reproducing in these areas were *Arthroleptis* species, which have direct development (Guibé & Lamotte, 1958; Lamotte & Perret, 1963). The lower densities of *P. guineensis* in secondary forests may be due to lower densities of tree-holes with sufficiently long water holding capacity. Other reasons might be unsuitable habitats, with *e.g.* lower humidity and higher temperature.

Breeding sites were only visited for reproduction. During night both males and females were absent at breeding sites. We do not know where adults and juveniles live when they are not present at breeding sites. Field observations showed that juveniles of several *Phrynobatrachus* species were more abundant in drier than in more humid parts of the forest (Rödel and Ernst, unpubl. data), suggesting that drier parts might offer advantages to small frogs (*e.g.*, higher abundances of small prey or lower predation pressure). Hence, it would be beneficial for the extremely small juveniles of *P. guineensis* when reproduction occurs in drier parts of the forest.

Activity and lifespan. Reproductive activity of *Phrynobatrachus guineensis* was highly seasonal. However, activity variables like presence of males at breeding sites, number of used breeding sites and number of clutches were not correlated with rainfall, if analyzed for the whole study period. However, in the comparatively dry months, from October 2001 to September 2002, clutch number and precipitation was correlated. Most likely *P. guineensis* triggers reproduction according to several factors. In wet years it might not be necessary to rely on rainfall patterns, because of its high frequency and amount and the resulting high water holding capacity of breeding sites. In dry years reproduction might be only successful when closely linked to a certain minimum amount of precipitation. *Phrynomantis microps*, a West African savanna dwelling microhylid, likewise starts reproduction in dry years only after a certain amount of rain has fallen, but reproduces after every rain in wet years (Rödel, 2000a). Lower minimum survival rates in 2001 support the idea that this was an exceptionally bad year for *P. guineensis*. The fact that calculated maximum population size was highest in 2001 might thus be an artifact of low recapture rates due to higher mortality.

Phrynobatrachus accraensis reaches sexual maturity within four to five months and survives only two more months (Barbault & Trefaut Rodriguez, 1979b; Barbault, 1984). With a mean of about one month, the recorded reproductive lifespan of male *P. guineensis* was even less. Only a very small proportion of the adult population survived the dry season. It is likely that *P. guineensis* can reproduce within the year of metamorphosis, which is also known for several other West African frogs (*Arthroleptis peocilonotus*: Barbault & Trefaut Rodriguez, 1979a; *Phrynobatrachus* spp.: Barbault & Trefaut Rodriguez, 1979b, Barbault & Pilorge, 1980; Barbault 1984; *Hyperolius nitidulus*: Lampert & Linsenmair, 2002). In contrast to the latter species, estimated population size in *P. guineensis* remained more or less constant throughout the three years of investigation. Although at least some males and females seem to live long enough to reproduce more than once, it is likely that a single clutch is a considerable part of a female's lifetime reproduction investment.

Breeding site selection. There are several abiotic factors that could negatively affect tadpole development, growth, and survival (Smith-Gill & Berven, 1979; Wilbur, 1984; Travis & Trexler, 1986; Warner *et al.*, 1991), and might therefore affect oviposition site selection by either males, females or both. Some studies showed that water depth, potentially a cue for

water persistence, was important for spawning site choice (Crump, 1991; Kuhn, 1992; Ildas & Ancona, 1994). Spieler & Linsenmair (1997) reported that clutch size deposited by a particular *Hoplobatrachus occipitalis* female was positively correlated with water holding capacity of the pools, and that females could assess this property by visiting pools during a season repeatedly, or by using information already gained during previous seasons.

Phytotelmata often have low oxygen concentrations (Lannoo *et al.*, 1987; Kitching, 2000), or are acidic (Diesel & Schubart, 2000, Jungfer, 2000). The water persistence varies considerably across tree-holes and thereby influences food level, desiccation frequency, and predation (Kitching, 2000). Spawning frogs might frequently face a trade-off between the costs and the benefits of different sizes of tree-holes (see Smith, 1983, for similar trade-off in minute ponds). Small tree-holes desiccate faster and have lower nutrient levels (Apsbury & Juliano, 1998), but large tree-holes are less abundant and more likely to support predators (Fincke, 1992b; Rödel *et al.*, 2003).

In this study suitable breeding sites varied across time and space. Rainfall and therefore desiccation of tree-holes was unpredictable. Breeding sites that have been successfully used in one year could become unsuitable in the next because of rotting wood or new holes punctured in fruit capsules and snail shells. In combination with a short adult lifespan, *Phrynobatrachus guineensis* thus will not be able to establish traditional spawning sites. This assumption is consistent with the high turnover rates in breeding sites observed between seasons.

Often males stayed only one day at a certain tree-hole. It is possible that they evaluated different sites for suitability and just remained longer at those that provided better reproductive chances. In most cases when females were observed at a breeding site, we did not find new clutches the same or following day, suggesting that females inspect and evaluate males, breeding sites or both, before eventually spawning.

Staying within a certain area is a necessary requirement allowing continuous inspections and evaluations of potential breeding sites. Inhabited parts of the forest showed a clumped pattern of used breeding sites. This clumped pattern might be comparable to lek breeding/chorus calling found in other frog species, facilitating attraction of potential mates. Often males seemed to tolerate other males at the same site. Possible reasons may be that occupation of the same or neighboring holes by many males may increase chances to attract females, or that within an area breeding sites are limited (Donnelly, 1989a,b). However, the costs may be increased competition for mates and lowered fertilization rates due to disturbances by other males. The longest registered migrating distances in *Phrynobatrachus guineensis* were 80-90 m. Thus, most individuals probably remain in a given area of only a few hectares. The small size limits the dispersal ability and might also explain why *P. guineensis* was not using all potentially suitable breeding sites.

Larval development and mortality. Large tree-holes provide more food and space for tadpoles, and thus one might have presumed these to be preferable to *Phrynobatrachus guineensis*. However, *P. guineensis* was absent from large tree-holes, which usually contained predatory dragonfly and fly larvae and/or *Acanthixalus sonjae* tadpoles. Dragonfly and fly larvae belong to the few common predators recorded from phytotelmata (Fincke, 1992a; Mogi & Yong, 1992; Kitching, 2000; Yanoviak, 2001; Clausnitzer, 2002; Clausnitzer & Lindeboom 2002). Experiments showed that tadpoles did not survive in the presence of dragonflies, but developed normally and metamorphosed successfully if predators were removed. Caldwell (1993) likewise found that survival of different phytotelmata dwellers, involving predatory insects and anurans, depended on assemblage composition and species phenology. It is known that some species can detect predators and respond adaptively by avoiding these habitats (Resetaris & Wilbur, 1989; Hopey & Petranka, 1994; Spieler & Linsenmair, 1997), whereas in others there was no such evidence (Laurila & Aho, 1997). In this study, oviposition was recorded five times in two tree-holes containing dragonfly larvae. However, it remains unclear if this was a "mistake" made by some females or if *P. guineensis* cannot detect dragonfly larvae. It is possible that *P. guineensis* avoids predation indirectly by avoiding large tree-holes.

Desiccation follows predation as a major factor in tadpole mortality in temporary habitats (Smith, 1983; Alford & Wilbur, 1985; Wilbur, 1987; Newman, 1988; Laurila & Kujasalo, 1999) and was shown to be an important mortality factor also in this study. *Phrynobatrachus guineensis* deposits much smaller clutch sizes than other species of the genus (Wager, 1986; Rödel, 2000a). However, its eggs are relatively larger and richer in yolk. Embryonic and larval development was completed within three weeks. Such rapid development is generally seen as an adaptation to the desiccation risk in short-lived habitats (Newman, 1992). Other *Phrynobatrachus* that reproduce in small puddles show also shorter developmental periods (Rödel & Ernst, 2002b) than *Phrynobatrachus* reproducing in larger water bodies (Wager, 1986; Rödel, 2000a). The risk of desiccation varied strongly not only among tree-holes but also within a tree-hole during the rainy seasons. Tree-holes with a high water level or large volume after rainfall sometimes lost water faster than smaller tree-holes (Rudolf & Rödel, unpubl. data). Thus, total values of water depth or volume were not reliable measurements for water persistence. Under such variable conditions there should be a strong selective pressure on the ability of *P. guineensis* to estimate the water holding capacity of possible oviposition sites. Recent observations suggest that *P. guineensis* indeed chooses breeding sites at least partly with respect to water persistence (Rudolf & Rödel, unpubl. data). However, about a quarter of tadpole mortality still was due to desiccation.

Another biotic factor that could affect developmental

success is food availability (for summary see Alford, 1999). Food is assumed to be limited for tadpoles in phytotelmata (Jungfer, 2000). The reasons are repeated drying up of these breeding sites (Apsbury & Juliano, 1998) and the often low primary production due to limited light and the shape and position of holes, often preventing nutrition influx (Kitching, 2000). This is seen as the major factor causing the evolution of highly sophisticated parental care in several phytotelmata dwelling anurans, like individual transport of tadpoles to separated sites or supplying tadpoles with nutritive eggs (e.g., Weygoldt, 1980; Duellman & Trueb, 1986; Jungfer, 1996; Jungfer & Weygoldt, 1999; Chen *et al.*, 2001, Schiesari *et al.*, 2003). We have no indication of any kind of parental care in *P. guineensis* other than choosing suitable breeding sites. Lack of parental care is also indicated by the high mortality of eggs. Tadpole densities observed in this study are the highest ever reported (compare *e.g.*, Bragg, 1940; Heyer, 1973; Wilbur, 1977; Semlitsch & Caldwell, 1982; Woodward, 1982; Smith, 1983; Newman, 1989; Kam *et al.*, 1996, 2001; Rödel, 1998b). Therefore the large percentage of unexplained tadpole mortality might be due to a high level of competition. However, results of other studies indicate that competition is of comparatively minor importance for successful tadpole development (Rudolf & Rödel, unpubl. data). Tadpoles of the highest density successfully completed metamorphosis, and overall egg and tadpole mortality rates were low, compared to published data in other anuran species (*e.g.*, Calef, 1973; Licht, 1974; Kadel, 1975; Banks & Beebee, 1987, 1988; Riis, 1994; Barandun & Reyer, 1997; Spieler & Linsenmair, 1997; Rödel, 1998b).

Future Directions. In rare cases other *Phrynobatrchus* species might use tree-holes for reproduction, *e.g.*, *P. calcaratus* (Rödel & Agyei, 2003). However, these species seem to be much less well-adapted to this habitat by depositing large clutches that float on water. Future investigations are necessary to clarify the phylogeny within *Phrynobatrachus* and thus indicate an evolutionary pathway from aquatic eggs (Wager, 1986; Rödel, 2000a), to terrestrial clutches (Rödel & Ernst, 2002b), to tree-hole breeders with terrestrial clutches and finally to direct development (Rödel & Ernst, 2002a).

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ORIGIN OF A COMPLEX REPRODUCTIVE TRAIT: PHYTOTELM BREEDING IN MANTELLINE FROGS

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ABSTRACT

Many lineages of anurans have evolved novelties for breeding in plant-held water bodies (phytotelmata) and some authors have speculated on the origin of this trait. One hypothesis suggests that pond breeding was the ancestral condition of obligate phytotelm-breeding frogs, while another speculates that stream breeding was the ancestral condition. It is also possible that phytotelm breeding may have originated from other ancestral conditions such as direct development or phytotelm breeding itself may be the ancestral condition. None of these hypotheses, however, have been explicitly tested. To examine the origin of this trait, we adopted a phylogenetic approach using the endemic Malagasy frogs from the genera *Mantidactylus* and *Mantella* (Anura: Mantellidae). Six known species in *Mantidactylus* and one in *Mantella* are obligate phytotelm breeders. The remaining species in this diverse lineage include pond breeders, stream breeders and direct developers. We sequenced 1791 bp of the 12S, 16S and tRNA mitochondrial genes from twenty-six species of *Mantidactylus* and six *Mantella* including nearly all phytotelm-breeders and most putative species groups. Our well-supported phylogenetic hypothesis indicates that phytotelm breeding in mantelline frogs evolved twice independently (once in a monophyletic *Mantella* and once in a polyphyletic "*Mantidactylus*"). All phytotelm breeding "*Mantidactylus*" share a common ancestor and are sister to a pond breeding clade. Phytotelm breeding *Mantella* (*M. laevigata*) are also apparently descended from pond breeding ancestors. One reversal back to pond breeding has occurred ("*M*". *liber*) and in "*Mantidactylus*" obligate phyotelm breeding was preceded by facultative phytotelm dwelling.

Key Words: phytotelmata, character evolution, character origin, frog, phylogeny, reproductive mode, *Mantidactylus*, *Mantella*, Madagascar

INTRODUCTION

Frogs exhibit the greatest diversity of reproductive modes among vertebrates (Duellman, 1985). These modes of reproduction include the familiar aquatic egg deposition with pond-dwelling larvae as well as gastric brooders, foam nest builders, direct developers, and even truly viviparous species (Duellman & Trueb, 1986). These modes of reproduction are most diverse in tropical areas and many have been converged upon independently in different lineages. However, the direction, frequency and sequence of evolutionary changes that took place leading to this incredible diversification of anuran reproductive modes are poorly known.

One particularly interesting mode of reproduction among anurans is the use of phytotelmata for breeding. Phytotelmata are plant-held water bodies (*e.g.*, bromeliad tanks, treeholes, leaf axils, bamboo stumps, empty husks of nuts) that tropical frogs from at least 9 families have independently colonized as breeding habitat (Lannoo *et al.*, 1987; Lehtinen *et al.*, this volume). Numerous modifications among larvae and adults are associated with breeding in these small, food-poor micro-aquatic environments. A few of these modifications include air breathing in tadpoles (Lannoo *et al.*, 1987) obligatory oophagy (*e.g.*, *Hoplophryne* (Noble, 1929); *Philautus* sp. (Wassersug *et al.*, 1981), cannibalism (*e.g.*, some *Dendrobates* Caldwell, 1993; Caldwell & Araújo, 1998; Summers, 1999) and an array

of derived parental care behaviors (*e.g.*, Summers *et al.*, 1999; Lehtinen & Nussbaum, 2003). Eggs are laid either directly in phytotelmata or laid terrestrially and subsequently transported to these habitats. Development may be direct (*e.g.*, *Platymantis vitiensis*; Gibbons & Guinea, 1983) or free-living larvae may complete development inside the phytotelm. Larvae may be feeding (*e.g.*, *Osteopilus brunneus*; Lannoo *et al.*, 1987) or non-feeding (*Syncope antenori*; Krügel & Richter, 1995).

To understand the sequence of evolutionary events that led to phytotelm use by frogs, we need to know the ancestral condition (*i.e.*, its historical origin). Several authors have speculated that either pond or stream-breeding frogs were the most likely ancestors of phytotelm breeders (Duellman, 1985; Duellman & Trueb, 1986). For example, Duellman (1970) suggested that bromeliad-breeding hylids in the *Hyla bromeliacia* group "diverged from the lowland pond-breeding *picta*-stock by adapting to arboreal breeding habits in a successful attempt to invade the foothills in low mountains where ponds are scarce" (p. 681). In contrast, Wassersug (1980) noted similarities in larval morphology between the *H. bromeliacia* group and the *H. miotympanum* group (stream breeders), and concluded that phytotelm breeding evolved from stream breeders in this lineage. Wassersug (1980) also noted that a species closely related to the *H. miotympanum* group (*H. thorectes*) lays its eggs on vegetation above streams and speculated that "the ancestor of the present bromeliad breeding *Hyla* went through just such an evolutionary stage, first leaving eggs above water, that dropped into the water after hatching, then later developing tadpoles that could survive on vegetation." (p. 127). This hypothesized transition

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from stream breeding to bromeliad breeding is particularly satisfying because it explicitly includes a transitional stage and is congruent with the larval morphology.

Other origins for phytotelm-breeding are also possible. For example, within a particular lineage, phytotelm breeding itself might be the ancestral reproductive mode. Also, phytotelm breeding may have arisen independently more than once in a given lineage, possibly from different ancestral conditions. Another possibility is that phytotelm-breeding frogs may be descended from direct developing taxa. Intuitively, it may seem unlikely that species with free-living larvae would be *derived* relative to direct developing species but there is evidence for this scenario. Wassersug & Duellman (1984) presented evidence that some egg-brooding hylids with tadpoles evolved from species with direct development, possibly through heterochronic processes (see also Duellman *et al.*, 1988). This example demonstrates that our conception of what is derived and what is ancestral cannot be inferred in orthogenetic fashion.

An additional hypothesis for the evolution of phytotelm breeding (not mutually exclusive from those discussed above) is that obligate phytotelm breeding is preceded by a facultative phytotelm dwelling stage. Many non-phytotelm breeding frogs can be found in these habitats, particularly during dry periods. For example, in rainforests in Madagascar at least 16 species of non-phytotelm breeding frogs have been found in the water-filled leaf axils of *Pandanus* plants (Lehtinen, 2002). This facultative stage may be a precursor to obligate phytotelm breeding.

Compelling arguments can be made for a variety of conjectures concerning the evolution of phytotelm-breeding in anurans. However, it must be emphasized that these are only hypothetical explanations of the origin of this trait and none of these hypotheses have been explicitly tested. Fortunately, the validity of these hypothesized evolutionary sequences can be tested by adopting a phylogenetic approach.

Knowledge of phylogenetic relationships is useful in several ways in comparative biology. Frequently these data are used to test hypotheses of ancestral character states and their subsequent sequence of change (*e.g.*, Brooks *et al.*, 1995; Caldwell, 1996a; Brooks & McLennan, 2002). This phylogenetic perspective has been adopted in several previous studies of anuran evolution. For example, Caldwell (1996*b*) used information about evolutionary relationships in dendrobatids to study of the evolution of myrmecophagy in *Dendrobates*. Also, Emerson & Berrigan (1993) and Emerson (1994, 1996) used phylogenetic information to examine the processes by which southeast Asian fanged frogs (*Rana*) lost secondary sexual characteristics (see also Summers *et al.*, 1999; Ryan & Rand, 1993, 1999; Vences & Glaw, 2001b). The strength of utilizing phylogenies in comparative biological studies is the independent frame of reference it provides to test hypotheses and assumptions (Brooks & McLennan, 2002). However, using phylogenetic information as an independent

frame of reference is sound only if phylogenies are robust, well-supported and built with as few *a priori* assumptions as possible. We utilized this phylogenetic approach to test hypotheses regarding the evolution of phytotelm breeding in several frogs endemic to the island of Madagascar.

Taxonomy, Classification and Hypotheses. The family Mantellidae currently contains 3 subfamilies: Boophinae (which includes the genus *Boophis*), Laliostominae (*Aglyptodactylus* and *Laliostoma*) and Mantellinae (*Mantidactylus* and *Mantella*; Vences & Glaw, 2001a). *Mantella* display a remarkable convergence with the neotropical dendrobatids including toxic skin alkaloids, diurnal activity, aposematic coloration and myrmecophagy (Vences & Glaw, 1999). *Mantella* currently includes 17 described species, one of which breeds in phytotelms. *Mantella laevigata* lays its eggs in water-filled bamboo stumps or treeholes and has oophagous tadpoles (Glaw & Vences, 1994; Heying, 2001). All other *Mantella* for which reproductive data are available lay eggs terrestrially at the margins of ponds or slow streams (Blommers-Schlösser, 1979; Blommers-Schlösser & Blanc, 1991; Glaw & Vences, 1994; Vences & Glaw, 1999). No *Mantella* are known to inhabit phytotelms facultatively (Lehtinen, 2002).

The genus *Mantidactylus* includes 69 described species (Frost, 2002) and exhibits a great range of reproductive diversity including pond breeders, stream breeders and direct developers (Glaw & Vences, 2000). In addition, there are five described and one undescribed species of *Mantidactylus* (*albolineatus*, *bicalcaratus*, *flavobrunneus*, *pulcher*, *punctatus* and sp. nov.) that live and breed in the water-filled leaf axils of plants (usually *Pandanus*). The five described species have been placed in the subgenus *Pandanusicola* on the basis of this similarity in breeding habitat (Glaw & Vences, 1994). Many other species of *Mantidactylus* are known to inhabit phytotelmata facultatively (Lehtinen, 2002). In all known cases, mantelline frogs do not lay eggs directly in water.

Past attempts to classify the mantelline frogs resulted in the phenetic species groups of Blommers-Schlösser & Blanc (1991) and the subgenera of Dubois (1992) and Glaw & Vences (1994). Several recent datasets have provided some tests of the relationships among and the monophyly of these groupings (*e.g.*, Bossuyt & Milinkovitch, 2000; Richards *et al.*, 2000; Vences & Glaw, 2001b; Glaw & Vences, 2002; Wilkinson *et al.*, 2002). Richards *et al.* (2000) included some phytotelm breeders and placed *M. flavobrunneus* (a phytotelm breeder) as sister to *M. depressiceps*[1] (a pond breeder). This clade was sister to *Mantella*. However, most species groups of *Mantella* and some species groups of *Mantidactylus* were not included in this analysis and those included were represented only by a single species. Further, as the phytotelm breeding *Mantella laevigata* was not included, it is impossible to know how the placement of this species would affect our interpretations of the origins of phytotelm breeding.

[1]This specimen (UMMZ 198114) has been re-identified as *Mantidactylus katherinae*).

By reconstructing the phylogenetic relationships of the mantelline frogs using an expanded dataset, we can test the following hypotheses regarding the origin of phytotelm breeding in this lineage:

Hypothesis 1: *Phytotelm breeding evolved once in mantelline frogs.* If the lineage containing all phytotelm breeding *Mantidactylus* and *Mantella* is monophyletic, then this trait arose only once. Conversely, if this lineage is not monophyletic then phytotelm-breeding arose independently more than once.

Hypothesis 2: *Phytotelm breeding evolved from pond-breeding ancestors.* If the immediate ancestors of phytotelm breeders are pond-breeders, we would conclude that this was the ancestral condition. However, stream-breeders or direct developers could also be the ancestors of phytotelm breeders. Also, it is possible that, within a given lineage, phytotelm breeding was the ancestral condition from which other reproductive modes subsequently evolved.

Hypothesis 3: *Facultative phytotelm dwelling is a precursor to obligate phytotelm breeding.* Facultative use of phytotelms may be a necessary step before obligate phytotelm breeding can evolve. Behavioral association with these plants would seem to make the origin of phytotelm breeding from these ancestors more likely. This hypothesis is supported if facultative phytotelm dwellers are ancestral to obligate phytotelm breeders.

MATERIALS AND METHODS

Forty taxa were included in this study, 32 ingroup and 8 outgroup (see Appendix A). The ingroup included 26 *Mantidactylus* and 6 *Mantella* (compared to 9 *Mantidactylus* and 2 *Mantella* in Richards *et al.*, 2000). This included multiple members of all putative *Mantidactylus* subgenera and at least one member from each putative species group of *Mantella* (except the monotypic *Mantella bernhardi* group, for which tissues were unavailable). Six of the seven known phytotelm-breeding species (*Mantella laevigata* and *Mantidactylus albolineatus, bicalcaratus, flavobrunneus, pulcher* and sp. nov.) were included (tissues for *M. punctatus* were unavailable). Outgroup taxa included five representatives of the other two putative mantellid subfamilies (Boophinae: *Boophis albilabris, B. madagacariensis, B. tephraeomystax*; Laliostominae: *Aglyptodactylus laticeps, Laliostoma labrosa*), plus one African ranid (*Tomopterna delalandii*), one hyperoliid (*Tachycnemis seychellensis*) and one rhacophorid (*Nyctixalus pictus* – see appendix A). Multiple outgroups were used as the higher relationships of mantellids remain unclear.

We assumed that phytotelm-breeding is a derived trait and that pond breeding (with aquatic eggs and larvae) is ancestral in Anura (Duellman, 1985; for another view see Bogart, 1980). This is justified on the basis of three observations: (1) a reproductive mode with lentic eggs and larvae is thought to be ancestral in salamanders and caecilians, (2) it is the most

phylogenetically widespread reproductive mode among anurans and (3) it is characteristic of most basal families (Duellman & Trueb, 1986). Within Mantellidae, the ancestral mode is also pond breeding (lentic eggs and larvae), as more basal taxa (*Aglyptodactylus, Boophis, Laliostoma*) are characterized by it (although some *Boophis* are stream breeders). In contrast, mantellines are characterized by terrestrial or semi-terrestrial egg deposition.

Molecular Methods and Phylogenetic Analyses. DNA was extracted from muscle, toe tissue or liver, and major portions of the mitochondrial 12S and 16S ribosomal genes plus the intervening tRNA^{val} gene were amplified. Products were then cleaned and sequenced as described in Richards *et al.* (2000).

Nucleotide sequences were aligned using ClustalX with the gap / extension penalties set at 10 / 5 (Thompson *et al.*, 1997). A second alignment was constructed from a reduced dataset after 300 bp of hyper-variable stem and loop areas were deleted. Sequences from the 12S, 16S and tRNA mtDNA (1791 bp total, 1491 bp for the reduced dataset) were combined in a total evidence analysis. We used maximum parsimony in PAUP* 4.0b10 (Swofford, 1998) to produce a hypothesis of phylogenetic relationships using heuristic searching with tree-bisection reconnection branch swapping. All characters were unordered and weighted equally. Gaps were treated as missing. Equally parsimonious trees were summarized by constructing a strict consensus tree. Bootstrapping (1000 replicates, using an identical search strategy) was used to assess the level of support for each node. More exhaustive branch and bound searches were used on various subsets of the full dataset. Habitat and reproductive characters were mapped onto the resulting consensus tree.

For comparative purposes, we also used maximum likelihood in PAUP* to assess phylogenetic relationships among these frogs. We used the Hasegawa-Kishino-Yano (HKY85) model with rate heterogeneity to parameterize the analysis. Empirical nucleotide frequencies, the transition – transversion ratio and the proportion of invariable sites were estimated by maximum likelihood. The rates for variable sites were assumed to follow a γ-distribution with shape parameter estimated by maximum likelihood. As with the parsimony analysis, we employed a heuristic search with tree-bisection reconnection branch swapping. Due to computational limits, bootstrap analyses were limited to 100 replicates. All sequences have been submitted to Genbank (Apendix A) and the alignment is available from RML or CMR.

To assess the monophyly of the clades of particular interest, we constructed constraint trees in PAUP* and repeated the heuristic search. In this search, only the most parsimonious trees in which the clade of interest was non-monophyletic were retained. The difference in the length of the most parsimonious trees where the clade was and was not constrained to be monophyletic gives an idea of how well supported the clade is. Constraint trees are analogous to Bremer support values.
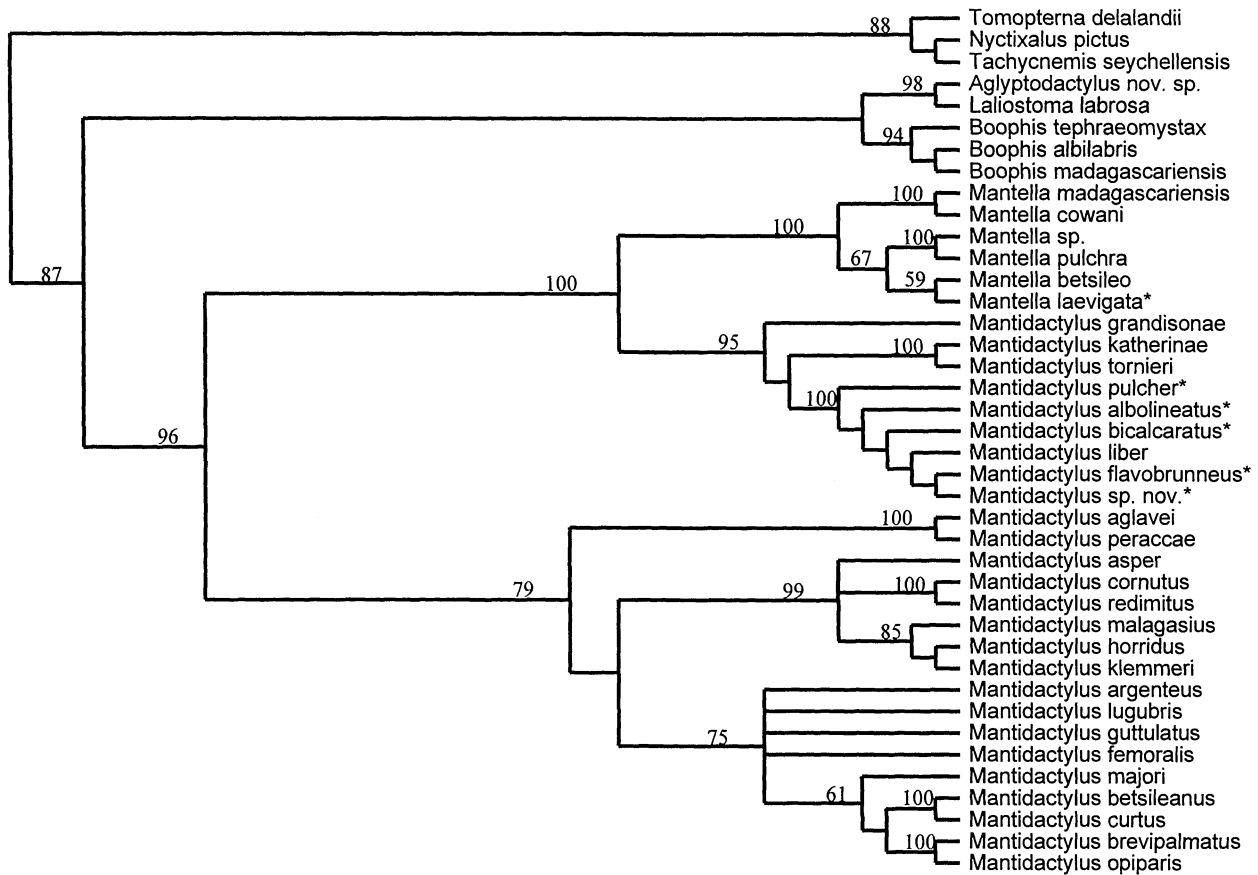
Fig. 1. Strict consensus tree of the four equally most parsimonius trees (5429 steps, CI = 0.295, RI = 0.466) using the full dataset (1791 bp, 835 informative sites) for all 40 species. Bootstrap support values (1000 replicates) are under each node. * indicates phytotelm breeder.

RESULTS

Analysis of 835 parsimony-informative characters produced four equally parsimonious trees of 5429 steps each (CI = 0.295 (excluding uninformative characters); RI = 0.466). The strict consensus of these four trees is presented in Fig. 1. These trees differed only in the placement some *Mantidactylus* in a region of the tree outside our focus here. Maximum likelihood analyses and parsimony analyses with the reduced dataset produced very similar trees (not shown). For the clades of interest, the topology was identical and herein we present the results of the parsimony analysis of the full dataset only.

These data effectively resolved relationships within and among the mantellid frogs. Most clades were well-supported and provide a firm basis for inference. The ranid, rhacophorid and hyperoliid outgroups were sister to all the mantellids (Fig. 1). Boophinae (*Boophis*) and Laliostominae (*Aglyptodactylus* and *Laliostoma*) form a clade that is sister to Mantellinae. This suggests a monophyletic Mantellidae (bootstrap support 87%). The mantellines were divided into two large, well-supported sister clades (bootstrap support 96%; Fig. 1). One of these clades includes the genus *Mantella* and a subset of *Mantidactylus*. The second large clade contains all the remaining *Mantidactylus*. All of the phytotelm-breeding mantellines are restricted to the former clade and further discussion is restricted to it. (Note: The genus *Mantidactylus* is polyphyletic; hereafter we use "*Mantidactylus*" to refer to the sister group of *Mantella*).

Within the *Mantella* + "*Mantidactylus*" clade, *Mantella* is monophyletic (bootstrap support 100%) and the phytotelm-breeding *M. laevigata* is relatively derived (Fig. 2). In "*Mantidactylus*," "*M.*" grandisonae is basal and sister to "*M.*" katherinae and "*M.*" tornieri (bootstrap support 95%; Fig. 2). This group is sister to the phytotelm-breeding "*Mantidactylus*" (bootstrap support 100%) which is monophyletic with the inclusion of "*M.*" liber (bootstrap support 100%). Within this clade, "*M.*" pulcher and "*M.*" albolineatus are relatively basal and "*M.*" bicalcaratus, "*M.*" flavobrunneus, "*M.*" liber, "*M.*" sp. nov are relatively derived (Fig. 2). The most parsimonious tree constructed with *Mantella* constrained to be non-monophyletic was 13 steps longer than when *Mantella* was monophyletic. The most parsimonious tree with "*M.*" liber placed outside of "*Mantidactylus*" was 14 steps longer than when this constraint was lifted.

Mapping reproductive data onto the strict consensus tree indicates that *Mantella laevigata* has several uniquely derived characteristics (phytotelm-breeding and oophagous tadpoles; Fig. 3). All other *Mantella* for which data are available have
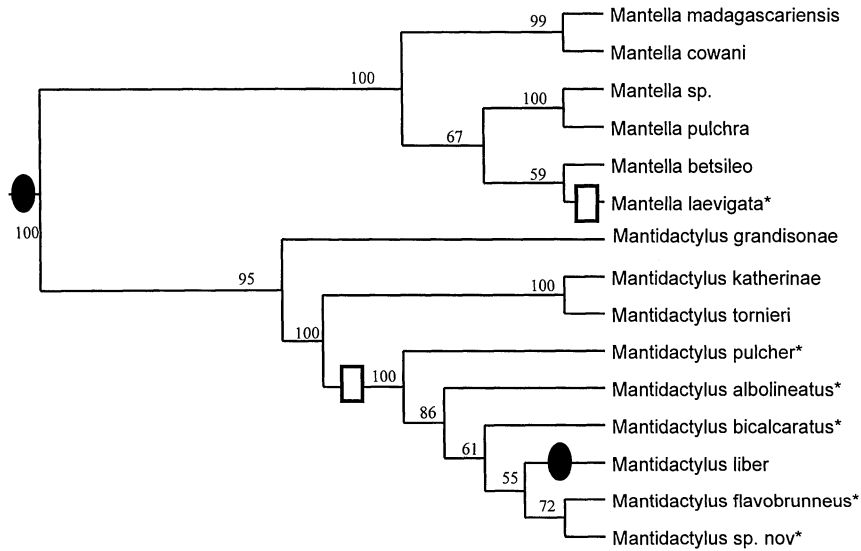
Fig. 2. A portion of the tree presented in Figure 1, including all the phytotelm-breeding species and their sister groups. Bootstrap support values (1000 replicates) are under each node. Dark ovals indicate pond breeders, clear boxes indicate phytotelm breeders. * indicates phytotelm breeder.

omnivorous larvae that develop in ponds or slow streams. In the "*Mantidactylus*" clade, all phytotelm breeding species are monophyletic (Fig. 2). "*Mantidactylus" liber* (a pond breeder) is strongly supported as a member of this clade. The phytotelm-breeding "*Mantidactylus*" (+ "*M." liber*) are sister to "*M*". *katherinae* and "*M*". *tornieri* which lay eggs on vegetation above ponds where the larvae complete their development (Fig. 4). This indicates that obligate phytotelm breeding arose twice independently in this lineage, both times from separate pond-breeding ancestors, with one reversal ("*M." liber*).

When information on facultative habitation of phytotelms is mapped onto the tree (Fig. 4) we see that phytotelm

utilization is uniquely derived in *Mantella laevigata* and other *Mantella* do not occupy these habitats facultatively. However, in "*Mantidactylus*" the sister group of the obligate phytotelm breeders (+ *M. liber*) are facultative phytotelm dwellers. In this lineage, it does appear that facultative phytotelm dwelling preceded the evolution of obligate phytotelm breeding (Fig. 4).

DISCUSSION

The Origin of Phytotelm Breeding. Obligate phytotelm breeding has evolved in at least 9 anuran families (Lehtinen et al., this volume), and thus there is great convergence in reproductive mode at the family level. However, within
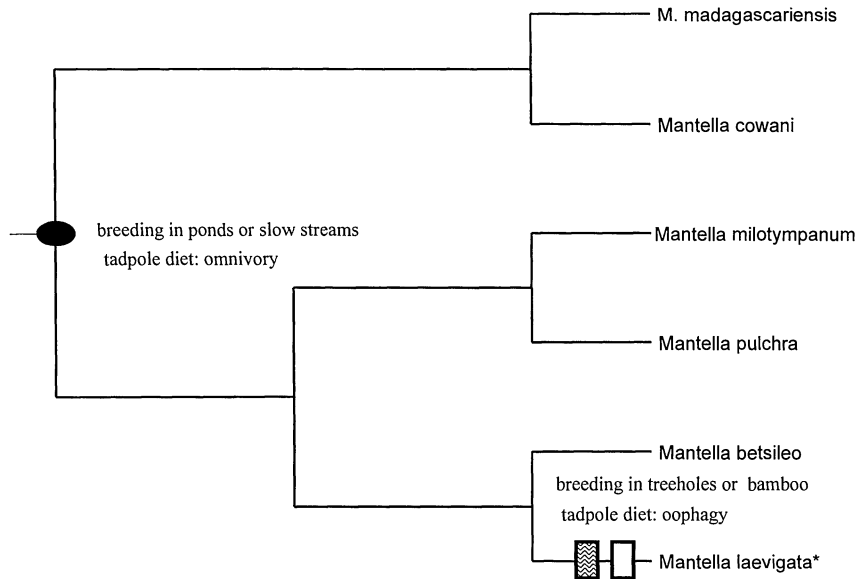
Fig. 3. Phylogenetic tree of *Mantella*, based on Figure 1. Dark ovals indicate omnivorous tadpoles in ponds or slow streams, clear and hatched boxes indicate oophagous tadpoles in bamboo stumps or tree holes, respectively. * indicates phytotelm breeder.

families, subfamilies and genera, evolutionary changes in reproductive mode exhibit less convergence and are more amenable to analysis. Our examination of the evolution of phytotelm breeding in the subfamily mantellinae (Anura: Mantellidae) indicates that: (1) this trait originated twice independently in this lineage (falsifying hypothesis #1), (2) in both cases, pond-breeding was the ancestral condition (failing to falsify hypothesis #2) and (3) in "*Mantidactylus*" (but apparently not in *Mantella*), this transition was correlated with facultative phytotelm dwelling in the ancestral sister group (falsifying hypothesis #3 in one case, but not in the other).

In *Mantella*, we are somewhat less certain that phytotelm breeding evolved from pond breeding ancestors than in "*Mantidactylus*." This is primarily because of the relative lack of information on breeding habits from natural populations. In addition to ponds, only slow streams are used for breeding, therefore, it is unlikely that stream breeding was ancestral in *Mantella*. Rather, pond breeding was probably ancestral and sluggish streams were secondarily colonized as these lotic habitats are the most similar to lentic ones.

Using only known taxa, we can't be sure that there is some undiscovered or extinct ancestor, with different reproductive characteristics. Also, we are implicitly assuming that species ancestral to the phytotelm breeders retain their ancestral reproductive mode. It is possible that these taxa may have subsequently evolved in other directions due to new selective pressures.

Nonetheless, given the conclusions presented above, a likely scenario by which phytotelm breeding originated in this clade is postulated as follows. Pond-breeding frogs first inhabited phytotelms facultatively (possibly for thermo- and/ or hydroregulatory purposes). Through some unknown series of changes in breeding behavior, females began laying egg masses on vegetation above water in phytotelmata, rather than on vegetation above ponds. Phytotelm breeding presumably conferred some selective advantage to these individuals compared to pond breeding. Their offspring survived and, as adults, also tended to breed in phytotelms. Phytotelm breeders had a minor radiation but, at some point, one species ("*M.*" *liber*) reverted back to the ancestral reproductive mode.

This scenario sounds like just another plausible hypothesis attempting to explain the origin of a putatively adaptive characteristic. The crucial difference is that this scenario was arrived at by hypothesis *testing*, not hypothesis *generation*. This sequence of evolutionary events was selected as the most likely origin of phytotelm breeding in this lineage based on well-supported phylogenetic information. However, the above scenario does not work as well for the origin of phytotelm breeding in *Mantella*. While pond-breeding does appear to be the ancestral condition, phytotelm breeding and oophagous tadpoles are uniquely derived in *M. laevigata*. As there are no known facultative phytotelm dwelling *Mantella*, nor other transitional forms, the specific details of the origin of phytotelm breeding in *M. laevigata* remain elusive.

Taxonomic Implications. There are several taxonomic changes suggested based on these data. First, the subfamilial groupings within Mantellidae (Vences & Glaw, 2001*a*) appear to be valid. *Aglyptodactylus* and *Laliostoma* (Laliostominae) are each other's sister group and the three *Boophis* (Boophinae) are monophyletic. These two clades are sister to the mantellines. However, as indicated by Richards *et al.* (2000), the genus "*Mantidactylus*" is polyphyletic, with the subgenera *Pandanusicola*, *Guibemantis* and *Blommersia* (part) being sister to *Mantella* rather than to other *Mantidactylus*. As previously suggested by Richards *et al.* (2000), a new genus will have to be erected to include these taxa. Also, "*M.*"
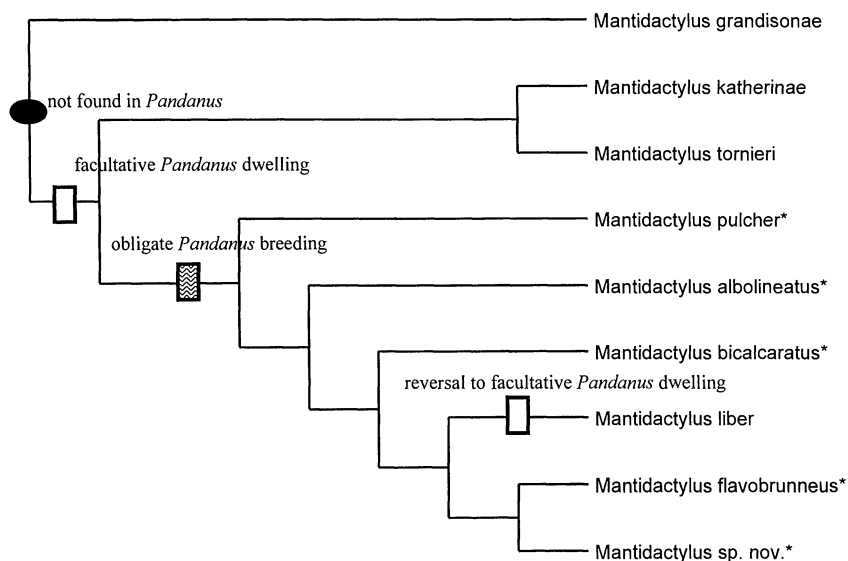

Fig. 4. Phylogenetic tree of "*Mantidactylus*", based on Fig. 1. Dark ovals indicate species not found in *Pandanus* plants. Clear boxes indicate species that are facultative inhabitants of *Pandanus* plants. Hatched boxes indicate obligate *Pandanus* breeding species. * indicates phytotelm breeder.

liber should be moved from the subgenus *Guibemantis* to the subgenus *Pandanusicola*, as the later subgenus is paraphyletic without its inclusion. Lastly, *M. argenteus* may not belong to the subgenus *Blommersia*, as it is not the sister taxon to "*M.*" *grandisonae*.

Conclusions. Herpetologists have long marveled at the staggering diversity of anuran reproductive modes and many have speculated on their origin (*e.g.*, Lutz, 1948; Goin, 1960; Goin & Goin, 1962; Poynton, 1964; Heyer, 1969; Coe, 1974; Crump, 1974, 1982; Duellman & Trueb, 1986; Magnusson & Hero, 1991). Speculation, however, does not advance our understanding of the evolution of these reproductive traits. Questions of trait origin are fundamentally historical questions and can only be reconstructed if the phylogenetic relationships are sufficiently known. Here, using a robust hypothesis of phylogenetic relationships, we have elucidated the direction, frequency and sequence of events that led up to the origin of phytotelm breeding in mantelline frogs. In this lineage, we found that this trait evolved independently twice (once in *Mantella* and once in "*Mantidactylus*") and the ancestral condition in both cases was pond breeding. Further, in "*Mantidactylus*," facultative phytotelm-dwelling was found to be a precursor to obligate phytotelm breeding. Whether the patterns uncovered here are similar for other lineages of phytotelm breeding frogs is unknown but clearly to rigorously address these questions a historical approach is necessary.

ACKNOWLEDGMENTS

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Appendix A. Taxa used in study. Species group assignments are from Blommers-Schlosser and Blanc (1991) and subgeneric classifications are from Dubois (1992) and Glaw and Vences (1994). * *Mantella* sp. nov of Richards et al. 1999. [+] from personal collection of A. Channing

Taxon	Family	Species group	Subgenus	Voucher Specimen	GenBank #
Nyctixalus pictus	Rhacophoridae	—	—	FMNH 231095	AF268254, AF268256
Tachycnemis seychellensis	Hyperoliidae	—	—	UMMZ 189347	AY454395
Tomopterna delalandii	"Ranidae"	—	—	AC1134[+]	AY454351, AY454372
Aglyptodactylus laticeps	Mantellidae	—	—	UMMZ 220339	AY454349, AY454370
Laliostoma labrosa	Mantellidae	—	—	UMMZ 213554	AY454350, AY454371
Boophis albilabris	Mantellidae	*albilabris* group	—	UMMZ 214050	AF261246, AF261264
Boophis madagascariensis	Mantellidae	*goudoti* group	—	UMMZ 198646	AF261248, AF261266
Boophis tephraeomystax	Mantellidae	*tephraeomystax* group	—	USNM 59146	AF026344, AF026360
Mantella betsileo	Mantellidae	*betsileo* group	—	UMMZ 211774	AY454373
Mantella cowani	Mantellidae	*cowani* group	—	UMMZ 211982	AY454360, AY454383
Mantella laevigata	Mantellidae	*laevigata* group	—	UMMZ 212041	AY454352, AY454374
Mantella madagascariensis	Mantellidae	*madagascariensis* group	—	UMMZ 197359	AF261234, AF261252
*Mantella milotympanum**	Mantellidae	*aurantiaca* group	—	UMMZ 212228	AF026353, AF026372
Mantella pulchra	Mantellidae	*madagascariensis* group	—	UMMZ 212140	AY454353, AY454375
Mantidactylus aglavei	Mantellidae	*aglavei* group	*Spinomantis*	UMMZ 212357	AF261236, AF261254
Mantidactylus albolineatus	Mantellidae	*pulcher* group	*Pandanusicola*	ZSM 250/2002	AY454354, AY454376
Mantidactylus argenteus	Mantellidae	*argenteus* group	*Blommersia*	UMMZ 212459	AY454377
Mantidactylus asper	Mantellidae	*asper* group	*Gephyromantis*	UMMZ 212503	AY454355, AY454378
Mantidactylus betsileanus	Mantellidae	*ulcerosus* group	*Brygoomantis*	UMMZ 197432	AF261237, AF261255
Mantidactylus bicalcaratus	Mantellidae	*pulcher* group	*Pandanusicola*	UMMZ 197485	AY454356, AY454379
Mantidactylus brevipalmatus	Mantellidae	*albofrenatus* group	*Chonomantis*	UMMZ 212681	AY454357, AY454380
Mantidactylus cornutus	Mantellidae	*granulatus* group	*Phylacomantis*	UMMZ 213328	AY454358, AY454381
Mantidactylus curtus	Mantellidae	*ulcerosus* group	*Brygoomantis*	UMMZ 212717	AY454359, AY454382
Mantidactylus femoralis	Mantellidae	*lugubris* group	*Ochthomantis*	UMMZ 197651	AF261238, AF261256
Mantidactylus flavobrunneus	Mantellidae	*pulcher* group	*Pandanusicola*	UMMZ 212922	AF261239, AF261257
Mantidactylus grandisonae	Mantellidae	*argenteus* group	*Blommersia*	UMMZ 212950	AY454361, AY454384
Mantidactylus guttulatus	Mantellidae	*guttulatus* group	*Mantidactylus*	UMMZ 213025	AF026352, AF026371
Mantidactylus horridus	Mantellidae	—	*Laurentomantis*	UMMZ 201404	AY454362, AY454385
Mantidactylus katherinae	Mantellidae	*depressiceps* group	*Guibemantis*	UMMZ 198114	AF261242, AF261260
Mantidactylus klemmeri	Mantellidae	*boulengeri* group	*Gephyromantis*	UMMZ 213033	AY454363, AY454386
Mantidactylus liber	Mantellidae	*depressiceps* group	*Guibemantis*	UMMZ 213093	AY454364, AY454387
Mantidactylus lugubris	Mantellidae	*lugubris* group	*Hylobatrachus*	UMMZ 213138	AY454388
Mantidactylus majori	Mantellidae	*lugubris* group	*Ochthomantis*	UMMZ 197906	AY454389
Mantidactylus malagasius	Mantellidae	—	*Laurentomantis*	UMMZ 211685	AY454365, AY454390
Mantidactylus opiparus	Mantellidae	*albofrenatus* group	*Chonomantis*	UMMZ 197996	AF261240, AF261258
Mantidactylus peraccae	Mantellidae	*aglavei* group	*Spinomantis*	UMMZ 213267	AY454366, AY454391
Mantidactylus pulcher	Mantellidae	*pulcher* group	*Pandanusicola*	UMMZ 213314	AY454368, AY454393
Mantidactylus redimitus	Mantellidae	*granulatus* group	*Phylacomantis*	UMMZ 198105	AF261241, AF261259
Mantidactylus sp. nov.	Mantellidae	—	—	FMNH 259923	AY454367, AY454392
Mantidactylus tornieri	Mantellidae	*depressiceps* group	*Guibemantis*	UMMZ 213353	AY454369

THE EVOLUTIONARY ECOLOGY OF PHYTOTELMATA USE IN NEOTROPICAL POISON FROGS

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ABSTRACT

We review research on the evolutionary ecology of phytotelmata-breeding in neotropical poison frogs (dendrobatids). Recent advances in systematic analysis make it possible to develop a preliminary hypothesis concerning the evolutionary relationships within the family Dendrobatidae. This phylogenetic tree suggests that the evolution of phytotelmata-breeding has been conservative, evolving only a few times and being restricted largely to the genus *Dendrobates*. We review ecological factors that could potentially have driven the evolution of phytotelmata-breeding. We then review how the evolution of phytotelmata-breeding has influenced aspects of dendrobatid life history, including parental care, mating strategies, and egg and clutch size. Finally, we discuss evidence linking patterns of phytotelmata use to dendrobatid abundance and community structure.

Key Words: reproductive strategy, pool, Dendrobatidae

INTRODUCTION

Research on the ecology and evolutionary biology of tropical anurans has lagged behind work on their temperate counterparts, although great strides have been made in the recent past (*e.g.*, Savage, 2002). Research on the evolution and ecology of phytotelmata use in tropical species has been especially sparse until recently. As this symposium volume demonstrates, the ecology and evolution of phytotelmata use provide a wealth of stimulating opportunities for research, an excellent source of data for comparative analyses, and a host of new and intriguing questions relevant to fundamental biological issues.

The Neotropical poison frogs of the family Dendrobatidae display a diversity of reproductive strategies involving phytotelmata that make them excellent subjects for research on current issues in evolutionary biology and ecology. For example, many species display complex forms of parental care associated with the use of small pools, and this has important effects on the nature and intensity of sexual selection (Wells, 1978; Summers, 1989, 1990, 1992*a,b*). This review will focus on the evolution of phytotelmata-breeding in dendrobatid frogs, and will explore several aspects of life history that were profoundly affected by that transition. As yet, relatively little research has been done on these connections, and many of the results discussed here are preliminary. Nevertheless, the range of life history strategies within the family offers opportunities to address a variety of interesting ecological and evolutionary questions.

MATERIALS AND METHODS

For the most part, this article reviews and analyzes previously published research in a comparative framework. However, we will present results from original research on phytotelmata use

in three species of poison frogs: *Dendrobates ventrimaculatus* from Amazonian Ecuador, and *Dendrobates minutus* and *Dendrobates auratus* from eastern Panama. Summaries of the methods used to study each of these species are presented in Appendix 1. For further details on methodology, see Summers (1999) and Summers *et al.* (2000). We also present comparative analyses of data on reproductive characteristics (*e.g.*, egg and clutch size) as they relate to phytotelmata-breeding, using data gathered from the literature.

RESULTS AND DISCUSSION

Evolutionary Relationships in the Dendrobatidae. Understanding phylogenetic relationships among the poison frogs is critical if we are to correctly infer patterns of trait evolution, such as phytotelmata-breeding. Silverstone's monographs (1975, 1976) established the framework for subsequent systematic treatments of the aposematic members of the Dendrobatidae. Charles Myers and John Daly have made numerous contributions to our understanding of the systematic relationships within the Dendrobatidae (*e.g.* Myers and Daly, 1976; Myers *et al.*, 1995). Myers *et al.* (1991) identified *Aromobates nocturnus* as the basal lineage to other members of the family Dendrobatidae. Despite significant efforts by Edwards (1971, 1974), research on the evolutionary relationships of the cryptic species within Dendrobatidae (placed in the genera *Colostethus, Mannophryne and Nephelobates*) has lagged behind research on the more brightly colored taxa, and the systematics of this group are controversial (Coloma, 1995).

Recent studies of poison frog systematics have utilized molecular phylogenetic analysis (Clough and Summers, 2000; Vences *et al.*, 2000; Symula *et al.*, 2001, 2003). Researchers have also begun to include members of the genus *Colostethus* (*sensu lato*) in molecular phylogenetic analyses (La Marca *et al.*, 2002; Vences *et al.*, 2003). The resulting phylogenetic hypotheses allow us to investigate the relationships between

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ecological factors and life history traits in an evolutionary context.

Figure 1 shows a composite phylogeny that is derived from several of the recent molecular phylogenetic analyses mentioned above. Most studies have supported similar phylogenetic tree topologies, which should enhance our confidence that they are identifying actual historical relationships. Some taxa that were not included in any molecular analyses were placed on the tree on the basis of previous systematic analyses using morphological characters. For example, samples of *A. nocturnus* were not available for molecular systematic analyses. However, a suite of morphological characteristics indicates that this species represents the basal lineage of the dendrobatids (Myers et al., 1991).

The Evolution of Phytotelmata-breeding. Aromobates nocturnus is a stream-dwelling, nocturnal frog that shares several key morphological features with other members of the Dendrobatidae (Myers et al., 1991). The basal position of this species suggests that ancestral dendrobatids lived and bred in or

along streams, and this is also consistent with the life histories of other closely related outgroups (Vences et al., 2003). Other dendrobatid species typically deposit eggs in the leaf litter, and then transport tadpoles to water in streams or small pools, such as those that form in fallen palm fronds. Derived deposition strategies in the family include the production of nidicolous, endotrophic tadpoles (e.g., Junca et al., 1994; Caldwell and Lima, 2003) and the use of small phytotelmata above the forest floor. The distribution of tadpole deposition sites on the dendrobatid phylogeny indicates a single shift from stream and/or terrestrial pool-breeding to breeding in phytotelmata above the forest floor (Fig. 1). Strictly speaking, pools in fallen palm fronds should be considered phytotelmata. However, in this paper we are contrasting the use of small pools raised above the forest floor to use of terrestrial pools and streams. Hence we will restrict our use of the term phytotemata to small pools that are raised above the forest floor.

A variety of reasons have been suggested for transitions from breeding in permanent pools or streams toward more
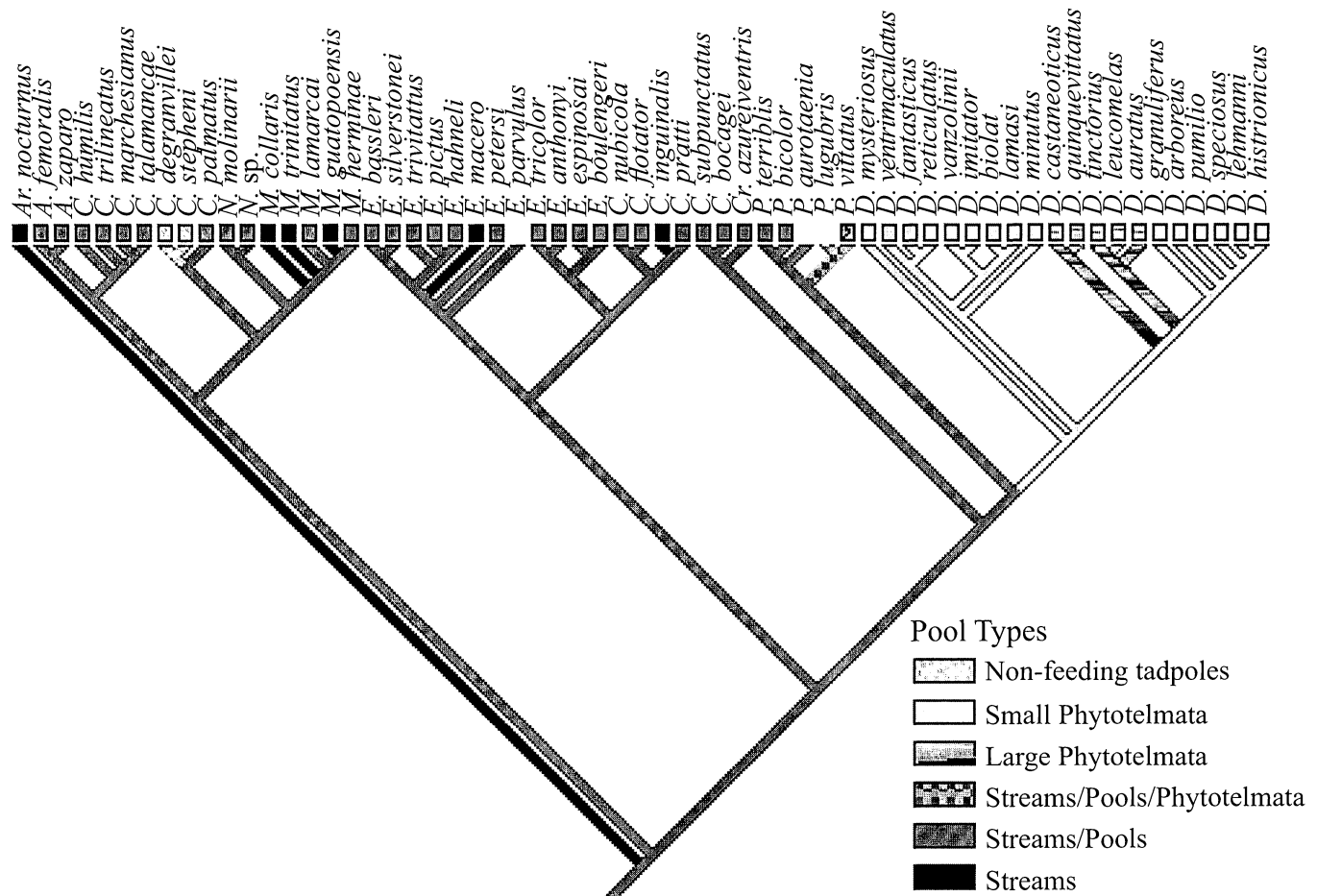
Fig. 1. Phylogenetic tree of dendrobatid frogs, compiled from recent systematic analyses. The nature of the water body utilized for breeding by each species is reconstructed (via squared change parsimony) on the tree. A few minor ambiguities in the reconstruction of breeding location evolution were resolved for the figure (using DELTRAN in MacClade), they do not affect the general conclusions. Data was not available for species without boxes under the species name. Generic abbreviations are as follows: Ar = *Aromobates*, A = *Allobates*, C = *Colostethus*, N = *Nephelobates*, M = *Mannophryne*, E = *Epipedobates*, Cr = *Cryptophyllobates*, P = *Phyllobates*, D = *Dendrobates*. The reconstruction was done with MacClade version 3.1 (Maddison & Maddison, 1993).

terrestrial reproductive strategies, such as phytotelmata-breeding (Crump, 1974; Harris, 1999). Predation by fish has been cited as a crucial factor (*e.g.,* Heyer *et al.*, 1975; Kats *et al.*, 1988), as has predation by invertebrates (*e.g.*, Formanowicz and Brodie, 1982). Magnusson and Hero (1991) proposed that egg predation by tadpoles is the most important factor favoring more terrestrial reproductive strategies by frogs in a central Amazonian site, and suggested that this factor is likely to have general importance. Other factors that could be important include parasitism, competition, and abiotic factors such as pool drying.

Testing the influence of particular factors hypothesized to have affected the evolutionary transition from stream and terrestrial pool breeding to phytotelmata-breeding is challenging because the transition occurred in the distant past. Reconstructing ancestral conditions is difficult, and carrying out evolutionary experiments on long-lived vertebrates is not usually feasible. Nevertheless, there are indirect ways of investigating evolutionary hypotheses. Streams, terrestrial pools and phytotelmata differ from each other in nature and density of predators, competitiors and parasites, and in abiotic factors such as size, dissolved oxygen, and temperature. Investigations of the effect of variation in these factors on the growth and survival of larvae, or on habitat choice by adults, across selected species spanning the evolutionary transition of interest can provide insight into which factors are likely to have had significant effects on egg and tadpole deposition strategies over evolutionary history (*e.g.*, Magnusson and Hero, 1994).

Numerous studies have investigated the effects of variation in biotic and abiotic factors on larval growth and survival in temperate ponds, revealing strong effects (*e.g.*, Wilbur, 1987). Many studies of temperate frogs have also investigated how biotic and abiotic factors influence habitat choice by adults. For example, Resetarits and Wilbur (1989) demonstrated the ability of female *Hyla chrysoscelis* to discriminate among oviposition sites (pools) on the basis of both potential predation and competition. Relatively little work on the effect of these factors on larval growth and survival or adult habitat selection has been done on dendrobatids. Below we review relevant research, and discuss how it contributes to our understanding of the evolution of phytotelmata-breeding.

Predation. The intensity of predation in the tropics, where most transitions to phytotelmata-breeding have occurred, is poorly understood and requires further investigation (Gascon, 1995; Harris, 1999). Even basic information on the distributions of potential predators in phytotelmata is limited. Fish are typically absent from small temporary, rain-filled terrestrial pools, as well as phytotelmata, although they do occur in large temporary pools (Wild, 1996). Invertebrate predators such as odonate and mosquito larvae are found across the Neotropics in large phytotelmata such as treeholes, and are known to prey on dendrobatid tadpoles (Fincke, 1992, 1994; K. Summers and C.S. McKeon, personal observations).

Hence, predators do occur in phytotelmata and can affect

larval survival. However, surveys at sites in Amazonian Ecuador, southern Peru, and in Panama suggest that these predators rarely colonize small phytotelmata, such as small treeholes, leaf and stem axils of plants, and bromeliad tanks (Summers, 1990, 1999; K. Summers, unpublished data). Other conspicuous invertebrate predators of terrestrial pools, such as dytiscid and hydrophilid beetles, also colonize phytotelmata above ground level rarely if at all (C.S. McKeon and K. Summers unpublished data). These results are consistent with research on predator density in small temperate pools. For example, Roth and Jackson (1987) demonstrated a lower rate of predation on *Hyla cinerea* tadpoles in small pools, due to lower colonization rates by predaceous insects. A low density of predators in phytotelmata may, in part, be due to their generally small size and consequent low nutrient levels.

Inter-specific predation may be rare in phytotelmata, but intra-specific predation (cannibalism) is likely to be common if multiple eggs and tadpoles are placed in a single pool, due to low nutrient levels and small size (Crump, 1992). Cannibalism is common in some species of *Dendrobates*. In *D. auratus*, 4-6 eggs are laid in the leaf litter and attended periodically by the male (Wells, 1978). After several weeks the males carry the tadpoles (usually one at a time) to phytotelmata, typically in tree holes (Dunn, 1941). Males typically carry the tadpoles from a single clutch to different pools, but will take tadpoles from different clutches to the same pool (Summers, 1990). Observations in the field and in the laboratory revealed frequent attacks, particularly by large tadpoles on smaller individuals (Summers, 1990; Caldwell and Araújo, 1998). Experimental manipulations demonstrated that cannibalism is the rule rather than the exception when tadpoles of this species occur together, and typically only a single tadpole survived in a pool (Summers, 1990). In *D. ventrimaculatus,* systematic monitoring of phytotelmata revealed frequent cannibalism of both eggs and tadpoles (eggs are placed just above the pool in this species). Experiments revealed that large tadpoles typically cannibalize smaller tadpoles, and that cannibalism enhances tadpole growth rates (Summers and Amos, 1997; Summers, 1999; Summers and Symula, 2001). Cannibalism also occurs in *D. minutus* (K. Summers, unpublished observations), and *D. castaneoticus* (Caldwell and Araújo, 1998). Although cannibalism may be common in dendrobatids breeding in phytotelmata, it is unlikely to have been an important deterrent to the transition from stream and terrestrial pool-breeding, because cannibalism would only have become a significant factor once the population density of phytotelmata-breeders was high, and because selection imposed by cannibalism is self-limiting (as cannibalism increases mortality, population density, and hence the probability of multiple deposition, is also likely to decline).

Can adult dendrobatids respond adaptively to the presence of predators or cannibals? Research on *D. auratus* in Panama and *D. ventrimaculatus* in Ecuador provides evidence for habitat selection with regard to egg and tadpole deposition. In

D. auratus, males carry tadpoles to pools far away from their territories, often depositing them in tree holes in the canopy. Males spend substantial amounts of time investigating pools before carrying tadpoles (Summers, 1989; Summers, 1990; Summers, 1992a), and will also investigate several pools during tadpole transport. Whether males evaluate pools on the basis of potential competitors or predators is unknown. In *D. ventrimaculatus*, systematic monitoring of pools revealed that the rate of egg deposition in *Heliconia* leaf axil pools declines dramatically once a tadpole has been placed in the pool. Experiments utilizing matching paired artificial axil pools to implement choice tests between axils with and without large tadpoles demonstrated that adults avoid both oviposition and tadpole deposition in pools containing tadpoles (Summers, 1999). Hence, *D. ventrimaculatus* adults are capable of evaluating the potential danger of cannibalism, and attempt to avoid it. In contrast, research on *D. castaneoticus* and *D. quinquevittatus* suggests that adults do not attempt to avoid depositing tadpoles in pools with larger tadpoles (Caldwell and Araújo, 1998).

"Basal" dendrobatid species, such as members of the genera *Colostethus*, *Allobates*, and *Epipedobates*, which typically release their tadpoles in small streams and terrestrial pools, are excellent subjects for comparative analyses of larval growth and survival and adult habitat choice. Investigations of habitat choice in these species can reveal which factors were likely to have influenced shifts in habitat use, such as the shift from breeding in terrestrial pools to phytotelmata.

Recent work in southeastern Peru with *Allobates femoralis* (C.S. McKeon and K. Summers, unpublished data) experimentally tested the tadpole deposition strategy of this frog in response to small terrestrial pools of different sizes and predator assemblages. In blocked, fully crossed tests, *A. femoralis* strongly preferred larger pools with caged belostomatids and consequent lowered levels of naturally colonizing dytiscid beetles (voracious tadpole predators), to small pools with and without belostomatids, and large pools with normal levels of beetle predators. These results indicate an important role for predation in pool choice in *A. femoralis*, and suggest that it may have played a significant part in the transition to phytotelmata-breeding. Further experiments on the effect of dytiscids on larval growth and mortality are certainly warranted for this system.

Parasitism. Kiesecker and Skelly (2000) have demonstrated the role of parasites in oviposition site selection in temperate treefrogs. The prevalence of parasites has not been investigated with regard to differences between streams, terrestrial pools and phytotelmata in the tropics. Intuitively it seems likely that parasites would be more common in streams and terrestrial pools, and could have been a factor favoring the transition to phytotelmata-breeding. This hypothesis awaits investigation.

Competition. Competition is another factor that may have favored the transition from stream or terrestrial pool-breeding to phytotelmata-breeding. Competition (both inter-specific

and intra-specific) is a common phenomenon among tadpoles in temperate pools, and both exploitative and interference competition have been demonstrated in field and laboratory experiments (Alford, 1999). Evidence for competition has also been found in tropical systems (*e.g.*, Heyer *et al.*, 1975; Inger *et al.*, 1986).

The streams and terrestrial pools utilized as tadpole deposition sites by members of *Epipedobates*, *Colostethus* and related genera are typically used by several different species of dendrobatids, and by other species of frogs as well (*e.g.*, Wild, 1996). In contrast, the number of species of frogs (dendrobatid or otherwise) using phytotelmata in a single area is typically low (*e.g.*, Summers, 1999). This trend has not been assessed quantitatively, but it is supported by general observations in a number of published studies, and by our own unpublished observations. If the tadpoles of frogs breeding in phytotelmata typically experienced less competition than those breeding in terrestrial pools or streams, this would favor a transition. The benefits (in terms of reduced competition) of breeding in phytotelmata are obviously frequency-dependent. As more species breed in particular phytotelmata, the more inter-specific competition is likely to occur. The effect may be particularly acute given the low nutrient levels associated with phytotelmata (Crump, 1992). This is intriguing, given the phylogenetic evidence presented above that the evolution of phytotelmata-breeding has been conservative. Once this ecological niche (phytotelmata) had been exploited by one evolutionary lineage, this may have largely prevented the independent evolution of similar strategies by other lineages due to competition. Comparative analysis of the levels of inter-specific and intra-specific competition experienced by tadpoles of both stream and terrestrial pool-breeding and phytotelmata-breeding dendrobatids is needed.

Some work has been done on intra-specific competition among dendrobatid tadpoles in phytotelmata. For example, field observations indicated that deposition of multiple tadpoles in single pools is common in *D. auratus* on Taboga Island, Panama (Summers, 1990). Experiments manipulating tadpole densities in phytotelmata used by *D. auratus* indicated that both exploitative and interference competition among tadpoles for resources negatively influenced growth rates (Summers, 1990). This could be seen as evidence against the hypothesis that competition drove a transition from streams or terrestrial pools to phytotelmata, but it should be noted that these levels of competition probably developed only after the ancestors of *D. auratus* had been breeding in phytotelmata for many generations. Presumably, levels of both intra-specific and inter-specific competition would be low in the early stages of the transition to phytotelmata-breeding.

Abiotic Factors: Pool Drying. Abiotic environmental factors also affect reproductive strategies. For example, many amphibian larvae face the threat of desiccation from pool drying. In *Pseudacris* treefrogs, there is an association between rates of growth and development and the probability of pond drying,

such that *P. triseriata*, which breeds in temporary ponds and faces a high threat of desiccation, reaches metamorphosis more quickly than *P. crucifer*, which breeds mainly in permanent ponds (Skelly, 1996). It is conceivable that differential risks of pool drying could have favored a transition from breeding in streams or terrestrial pools to breeding in phytotelmata, but there is little information available on the relative frequency with which phytotelmata dry out relative to streams or terrestrial pools.

A more basic question concerns whether or not dendrobatid tadpoles can respond adaptively to pool drying. Wilbur and Collins (1973) proposed a model integrating environmental influences on growth and development of anuran larvae. The model predicts that tadpoles will adjust their development to environmental factors affecting growth rate and pool duration. For example, they predicted that anuran larvae would respond to pool drying by accelerating their rate of development relative to growth, sacrificing large size at metamorphosis in order to leave the pond before it dries. Since their seminal paper, a variety of researchers have investigated how environmental factors influence growth and developmental trajectories in anuran larvae (*e.g.*, Wilbur, 1987; Tejedo, 1994). The results of these studies have been generally consistent with the Wilbur and Collins model (Alford, 1999).

A relatively small number of studies have focused on developmental plasticity in tropical systems (*e.g.*, Crump, 1989; Warkentin, 1995), and no one has worked on developmental plasticity in dendrobatids. There is ample evidence that seasonal patterns of rainfall and water-availability influence adult breeding patterns in the Neotropics (*e.g.*, Aichinger, 1987). Furthermore, small phytotelmata are subject to desiccation (*e.g.*, Summers, 1999). This suggests that studies of plasticity in response to pool drying regimes might be rewarding in dendrobatids. Here we present results from a study of the Amazonian poison frog, *D. ventrimaculatus* that suggest an adaptive association between pool drying and morphological changes during development.

Monitoring of pools (see Appendix 1) revealed that most

Heliconia axils pools lost water over time, eventually drying out and collapsing. Typically, this was a slow, gradual process, but small leaks can cause more rapid water loss. Most tadpoles were deposited in pools soon after their formation (Summers, 1999), and reached metamorphosis before the pools dried out and collapsed. However, some tadpoles were trapped in axils that had lost most of their water before they reached metamorphosis. They remained alive, but were unable to swim or (presumably) feed.

Tadpole weight was strongly related to both body length and total length (Linear Regression, for log body length on log weight: N = 37, R^2 = 0.81, t = 17.34, F = 150.74, P < 0.0001; for log total length on log weight: N = 37, R^2 = 0.75, t = 45.89, F = 103.22, P < 0.0001). An ANOVA on the residuals of the regression of log body length on log weight by stage category and final pool volume category (see Appendix 1) showed a significant main effect of pool volume, and a significant interaction effect of stage by volume (Fig. 2, N = 37, df = 1, F = 4.94 (volume), 0.002 (stage) and 4.22 (stage by volume); P < 0.05 (volume), P = 0.96 (stage), P < 0.05 (stage by volume)). This was not an effect of pool size, as there was no significant effect of pool size on the residuals of weight on log body length (Welch's Test, a one-way ANOVA robust to unequal variances), N = 42, P = 0.228). This was also not an effect of differences in the average stage of tadpoles taken from low and normal pools, as these were not significantly different within stage categories (t-test, N = 17, t = 0.261, P = 0.798, for late stage tadpoles).

These results indicate that late stage tadpoles in dry or almost dry pools tended to have a large body length for their weight. This could imply that late stage tadpoles in drying pools are accelerating development in order to reach metamorphosis before they desiccate. Alternatively, it could simply mean that these tadpoles are starving, and hence losing weight relative to their size. However, this latter hypothesis predicts that the same relationship seen for body length should hold for total length as well. This was not the case; there was a significant effect of pool volume on the residuals of log weight on log total length, but no significant effect of tadpole stage, nor any significant interaction between volume and stage (ANOVA, N = 37, df = 1, F = 9.29 (volume), 2.08 (stage) and 0.117 (stage by volume); P < 0.005 (volume), 0.159 (stage) and 0.734 (stage by volume)).

Although sample sizes are small, the evidence indicates that late stage *D. ventrimaculatus* tadpoles respond to pool drying by increasing their body length relative to body weight. This suggests that these tadpoles respond adaptively to pool drying by accelerating development (resorbing tail tissue). This hypothesis could be tested experimentally by randomly assigning late stage tadpoles from a single clutch to full or (nearly) empty pools (see Fincke, 1994). The larger issue of whether differential rates or probabilities of pool drying between terrestrial pools and phytotelmata have favored the transition to phytotelmata-breeding will have to await more
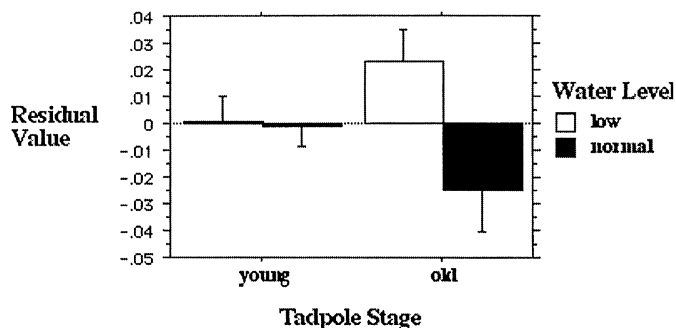
Fig. 2. Interaction plot of a Two-Way ANOVA on the residuals from the regression of tadpole weight on SVL. Factors are tadpole stage category (young = stage 25-35, old = stage 35-45) and pool water level category (low = less than 20 ml, normal = 20 ml or above). Error bars show one standard error.

data on pool drying (for both types of pools) in the field. Comparative studies of developmental plasticity in a variety of dendrobatid species would also be useful in this regard.

The Evolution of Complex Parental Care. The factors causing the transition to phytotelmata-breeding are (as yet) poorly understood, but there is considerable evidence that the transition had profound impacts on many aspects of dendrobatid life history. Parental care strategies, in particular, have been affected by the transition to phytotelmata-breeding, and these changes have in turn affected other reproductive strategies, such as courtship and competition for mates.

Most species of dendrobatids have male parental care, with periodic egg attendance and tadpole transport. In the *Dendrobates histrionicus* clade, female parental care predominates. In some species in this group (*e.g. D. pumilio*) the male periodically tends the eggs (Weygoldt, 1980), but the female transports them to phytotelmata. Females return regularly to the pools and provide trophic eggs for the tadpoles (Weygoldt, 1980; Brust, 1993). In other species (*e.g. D. histrionicus*) females perform all parental care duties (Weygoldt, 1987). However, even in species with biparental care (*e.g. D. pumilio*), the amount of time and effort that males put into parental care is small relative to that of females (Pröhl and Hödl, 1999). This has important implications for sexual selection (see below). In another clade of dendrobatids in Amazonia (*D. vanzolinii* and probably its close relatives), males and females form lasting pair bonds, and cooperate in parental care (Caldwell, 1997; Caldwell & Oliviera 1999). Males carry the tadpoles, and females provide trophic eggs for them periodically. Hence, in these species there is a more equitable distribution of parental effort between the sexes.

The most parsimonious reconstruction of male care on the poison frog phylogeny shows male care as primitive (Fig. 3), with female care evolving independently two times in the toxic dendrobatids: to biparental care in some Amazonian frogs (*Dendrobates vanzolinii* and close relatives), and asymmetric biparental and then uniparental female care in the *D. histrionicus* clade in northern South America and Central America.

The reasons for the transition to trophic feeding behavior (in either clade) are unknown. One possibility is that selection pressures such as those discussed above (*e.g.* predation and inter-specific competition) favored the exploitation of small pools, but the low availability of nutrients favored the evolution of provisioning. Females may have been favored to carry out this behavior because they can readily produce eggs, and hence more efficiently feed tadpoles (Summers and Earn, 1999). Alternatively, uniparental female care may have been driven by an interaction between sexual selection and a cost of polygyny to females (Summers and Earn, 1999; Weygoldt, 1987). This hypothesis is discussed in more detail below.

Parental Investment and Sexual Selection. The relationship between parental investment and sexual selection is central to our understanding of mating systems (Trivers, 1972). Females

invest more than males in parental effort in most species (Andersson, 1994). This appears to be the case in members of the *D. pumilio* group (see above). High female parental effort correlates with intense male-male competition and female selectivity (Pröhl and Hödl, 1999). Comparative research on populations living in different areas indicates that the intensity of sexual selection is influenced by the availability and distribution of phytotelmata (Pröhl, 2002).

In species with male parental care, male mating effort can affect the quality of paternal care, which in turn can reduce female fitness (Andersson, 1994). Sexual conflict occurs when males pursue polygyny, reducing the quality of parental care they provide (Summers, 1992b). This imposes a cost on some or all of a male's mates, leading to female-female aggression and mate guarding by females. This cost can accrue via the effects of male parental care on larval interactions. Evidence for this hypothesis, which connects larval ecology, parental care, and sexual selection, has rarely been documented in the field (Fincke, 1992).

Previous research has demonstrated sexual conflict in two species: *D. auratus* and *D. leucomelas,* two species with male parental care that utilize medium sized phytotelmata (Summers, 1989; Summers, 1990; Summers, 1992a,b). Males in both species are territorial, and attempt to attract and mate with all females they encounter in their territories. Some females associate with particular males for prolonged periods and try to prevent other females from mating with them (Summers, 1989, 1992a). Intrasexual aggression is frequent and intense in both sexes (Summers, 1989, 1992a). In *D. auratus,* some females suffer a cost (reduced offspring growth rates and increased offspring mortality) when their mates care for the offspring of other females (because males will deposit offspring from different clutches in the same pool). Experimental manipulation of tadpole densities in artificially constructed phytotelmata (in calabash husks and in tree holes) demonstrated significant effects of increasing tadpole numbers on both growth and mortality (Summers, 1990). Hence, in *D. auratus,* there appears to be a connection between male parental care, phytotelmata-breeding and sexual conflict (Summers, 1992b).

Sexual Conflict and Parental Care. Despite substantial research on the effect of parental investment on mating systems and sexual selection (see above), little attention has been paid to the idea that sexual selection may in turn influence parental care evolution. The poison frogs provide an excellent system in which to investigate the influence of a cost of polygyny on the evolution of female care from male care.

Weygoldt (1987) proposed that a high cost of polygyny to female reproductive success drove the transition from male care to female care in an ancestor of the *D. histrionicus* clade, which he hypothesized to be derived from the *D. ventrimaculatus* clade. He argued that there is a high cost of polygyny in the *D. ventrimaculatus* clade, and that this high cost of polygyny existed in the ancestor of the *D. histrionicus* clade.

Recent research provides evidence that there is a high cost of polygyny in a population of *D. ventrimaculatus* in Amazonian Ecuador (Summers and Amos, 1997; Summers, 1999; Summers and Symula, 2001). Males undertake the parental care, and they also use small phytotelmata, typically in the stem axils of *Heliconia* plants. Eggs are deposited just above the waterline in the phytotelmata, where they develop. Some tadpoles are transported by the males, while others go into the pool below. In spite of the fact that adults attempt to avoid placing eggs or tadpoles in pools with tadpoles (see above), pools with eggs frequently have tadpoles in them (Summers and Amos, 1997; Summers, 1999). This usually occurs because males deposit tadpoles in pools that have eggs already. Although eggs are placed above the waterline, increases in water levels caused by rainfall cause them to come into contact with the water, where tadpoles can reach them. Eggs are frequently cannibalized, and experiments in manipulated *Heliconia* axil pools have demonstrated that cannibalism enhances the growth rate of cannibals (Summers, 1999).

In some cases, this is a form of reproductive parasitism, in that the reproductive effort of one individual (in the form of eggs) is consumed by the offspring of another individual. The frequency and nature of such reproductive parasitism depends on the relatedness of the eggs and the cannibals. If they are full siblings, then cannibalism is similar to trophic egg feeding. If they are half-siblings, then cannibalism is a form of reproductive parasitism. Because polygny is common and males control the transport of tadpoles (Summers and Amos, 1997), the most likely scenario is that the egg and the cannibal are paternal half-sibs. In this case, one of the males' mates (the mother of the tadpole) would experience a benefit from polygyny, and the other (the mother of the egg) would experience a cost. Finally, if the egg and the cannibal are unrelated, then one adult pair (the parents of the tadpole) would be practicing a form of reproductive parasitism on another pair (the parents of the egg).

Genetic analyses using microsatellite markers indicate that eggs and tadpoles deposited in the same pool are a mixture of full-sibs, half-sibs and non-relatives (Summers and Amos, 1997). Tadpoles will cannibalize both relatives and non-relatives (Summers and Symula, 2001). Hence, reproductive parasitism is occurring in some instances of cannibalism. This begs the question of whether such parasitism is intentional on the part of males that engage in it. It is possible that constraints on pool availability make it inevitable that deposition of multiple eggs and tadpoles in a pool will occur, resulting in cannibalism. On the other hand, it is possible that males actively seek out opportunities to parasitize the eggs of a mate or of another pair. This would be equivalent to forms of "brood parasitism" seen in birds and social insects. The resolution of this issue awaits further investigation.

Although the research described above provides some support for Weygoldt's first argument (a high cost of polygyny in the *D. ventrimaculatus* clade), mapping the evolution of parental care on the phylogeny of the poison frogs contradicts Weygoldt's hypothesis that female care in the *D. histrionicus* clade derived from an ancestral member of the *D. ventrimaculatus* clade (Figure 3): biparental care in the *D. ventrimaculatus* clade and female care in the *D. histrionicus* clade evolved independently. Nevertheless, the evolution of female care in the *D. histrionicus* lineage could still have occurred in the manner suggested by Weygoldt (1987). Summers and Earn (1999) carried out a game theoretical analysis to investigate the assumptions and implications of the hypothesis that a high cost of polygyny drove the evolution of female parental care. Simple models demonstrate that the evolution of a pure female care strategy due to a cost of polygyny is not feasible. The reason for this is that the cost of polygyny necessarily declines as the frequency of female care increases in the population, reducing selection for female care. However, Summers and Earn (1999) showed that the cost of polygyny can interact in a reciprocally catalytic manner with the cost of lost mating opportunities to males. As female care increases in frequency, the cost of lost mating opportunities also increases (that is, the reproductive returns on male mating effort increase as the number of females performing care increases). In turn, increased mating effort comes at the expense of male parental effort. The coevolutionary dynamic between the cost of polygyny to females and the cost of lost mating opportunities to males can drive female care to fixation in the population (Summers and Earn, 1999).

Life History Tradeoffs in Growth and Development. The transition to phytotelmata-breeding affected many aspects of dendrobatid life history beyond parental care and mating strategies. For example, clutch size and egg size differ according to both pool type (phytotelmata versus terrestrial pools and streams) and parental care type (male versus female). Here we examine egg and clutch size in stream and terrestrial pool-breeders relative to phytotelmata-breeders from a comparative perspective. Ideally, we would approach these issues using modern comparative methods (Felsenstein, 1985; Harvey and Pagel, 1991). Unfortunately, the evolution of phytotelmata-breeding was conservative (Fig. 1), so variation is confounded by phylogenetic similarity. Here we present simple statistical analyses without taking phylogenetic relationships into account. Given the small sample sizes, we used nonparametric tests to be conservative. We also mapped characters onto the phylogenetic tree of the dendrobatids, using parsimony. These analyses can suggest potentially interesting relationships between life history characteristics. However, proper comparative analyses will have to be carried out on a larger phylogenetic scale (broad enough to include substantial convergent evolution), in order to test the validity of these relationships.

In contrast to some previous studies (*e.g.*, Salthe and Duellman, 1973), there was no significant relationship between egg and clutch size across species (Spearman Rank Correlation, N = 30, Rho = 0.020, P = 0.913). There was
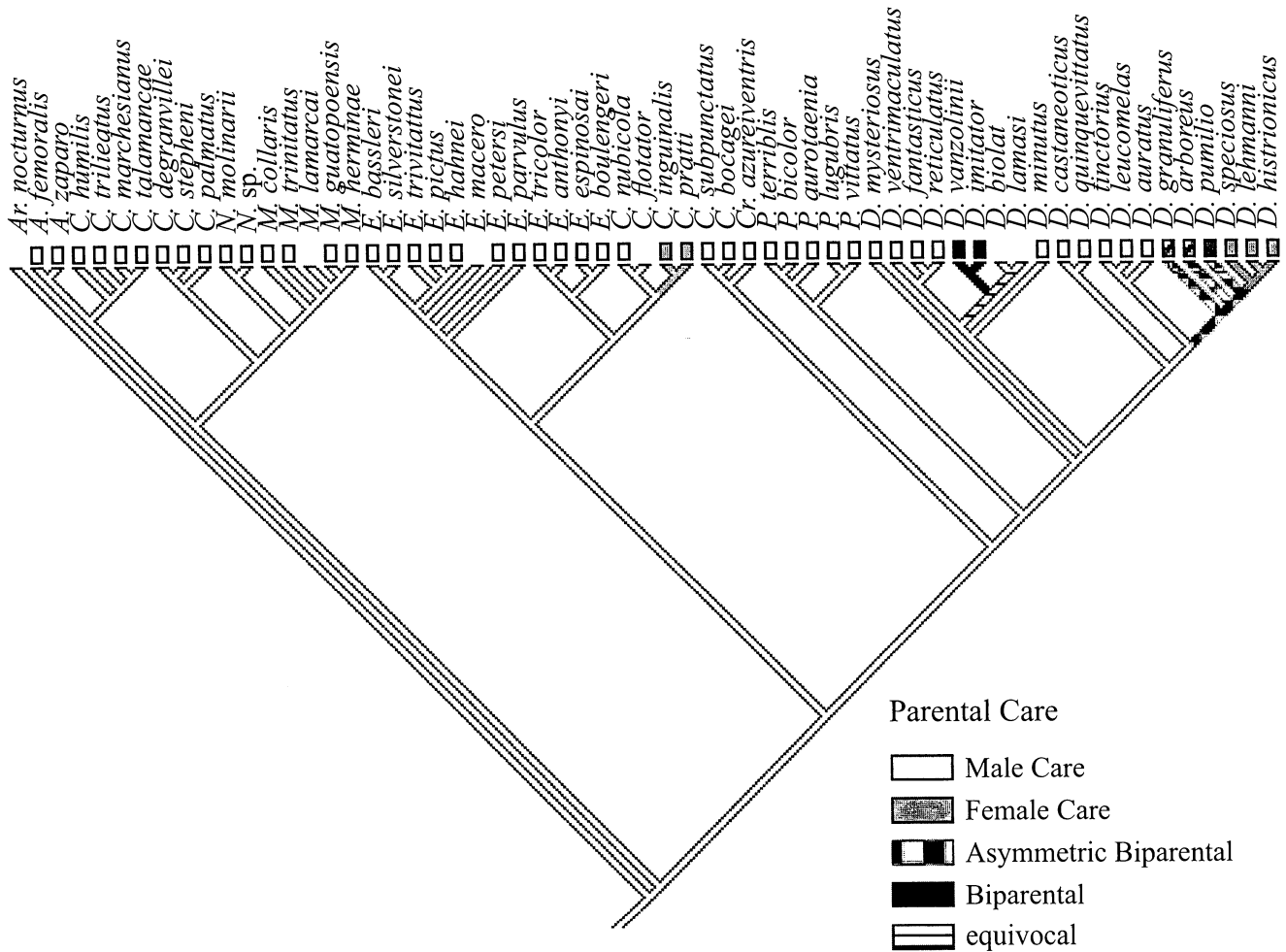
Fig. 3. The evolution of parental care, mapped onto the tree described for Fig. 1. Tree construction and abbreviations as in Fig. 1.

also no relationship between average female size and egg size (Spearman Rank Correlation, N = 29, Rho = 0.288, P = 0.128), or tadpole size (Spearman Rank Correlation, N = 23, Rho = 0.069, P = 0.747). There was, however, a significant relationship between clutch size and female size (SVL), as has been found in other taxa (Spearman Rank Correlation, N = 40, Rho = 0.549, P = 0.001). Using maximum female size yielded similar results. The direction of causality is not clear. Selection on clutch size may drive change in female size, or selection on female size in other contexts may secondarily influence the evolution of clutch size.

Clutch size declined with the transition from stream and terrestrial pool breeding to phytotelmata-breeding, and this appeared to be independent of the effect of female size on clutch size (Figure 4). Both factors could be examined simultaneously with a two-way ANOVA, but the variances of mean clutch sizes for stream and terrestrial pool breeders relative to phytotelmata-breeders were not homogeneous, violating an assumption of parametric ANOVA. Hence, we used separate Mann-Whitney U tests for species with small

(< 25 mm SVL) and large (≥ 25mm SVL) average female SVL. There was a significant decline in clutch size for each size category considered separately (Small size category: phytotelmata-breeders: N = 10, X = 3.84, SE = 0.48; stream/ pool breeders: N = 9, X = 13.01, SE = 1.80; Mann-Whitney U
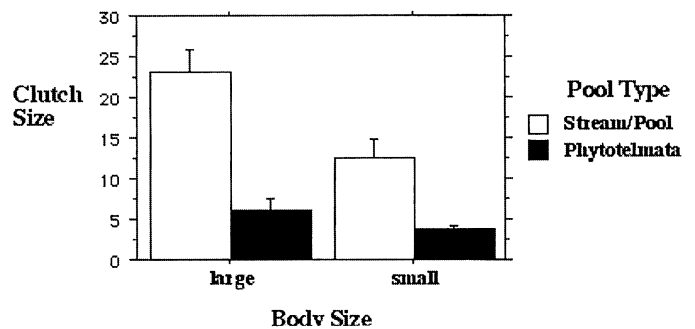

Fig. 4. Bar chart comparing clutch size in phytotelmata and stream/ pool breeding species, split by the average size of adults in the species (large = greater than or equal to 25 mm SVL, small = less than 25 mm SVL). Error bars represent one standard error.

= 5.00, P = 0.001; Large size category: phytotelmata-breeders: N = 5 = 7.66, SE = 1.91; stream/pool breeders: N = 15, X = 23.19, SE = 2.33; Mann-Whitney U = 3.00, P = 0.003). Figure 5 maps clutch size on the dendrobatid phylogenetic tree, illustrating the trend.

The reason for this trend is not known, and there are several possible explanations. For species with female parental care, the higher effort that locating and transporting individual larvae to phytotelmata (and feeding them) requires may cause a smaller allocation to embryo production as a result of a trade-off. This explanation is less likely to apply to species with male care, but females may adjust clutch sizes to provide a steady stream rather than large pulses of offspring for males to care for. This could reduce mortality by allowing males time to care for one set of offspring before another reaches maturity. A male caring for large numbers of offspring reaching maturity simultaneously (in a large clutch) may not be able to transport them all before they suffer from desiccation or predation. It is

also possible that selection for larger egg size in phytotelmata-breeding species with male care (see below) led indirectly to smaller clutch size due to inherent tradeoffs between egg and clutch size. We need to learn more about rates of clutch production in stream and terrestrial pool-breeders relative to phytotelmata-breeders in order to resolve the factors influencing clutch size.

There was no difference between phytotelmata-breeders and stream/pool breeders in egg size (phytotelmata: N = 11, X = 2.15, SE = 0.26; stream/pool: N = 17, X = 2.31, SE = 0.16, Mann-Whitney U = 81.50, P = 0.54), or in tadpole size (phytotelmata: N = 9, = 13.04, SE = 1.10; stream/pool: N = 11, X = 11.67, SE = 0.53, Mann-Whitney U = 34.50, P = 0.260). There is, however, a significant difference in egg size between species with male and female parental care (Fig. 6a, male care: N = 22, X = 2.43, SE = 0.15; female care: N = 4, X = 1.57, SE = 0.22, Mann-Whitney U = 13.00, P = 0.028). There is also a significant difference in tadpole size with regard to parental
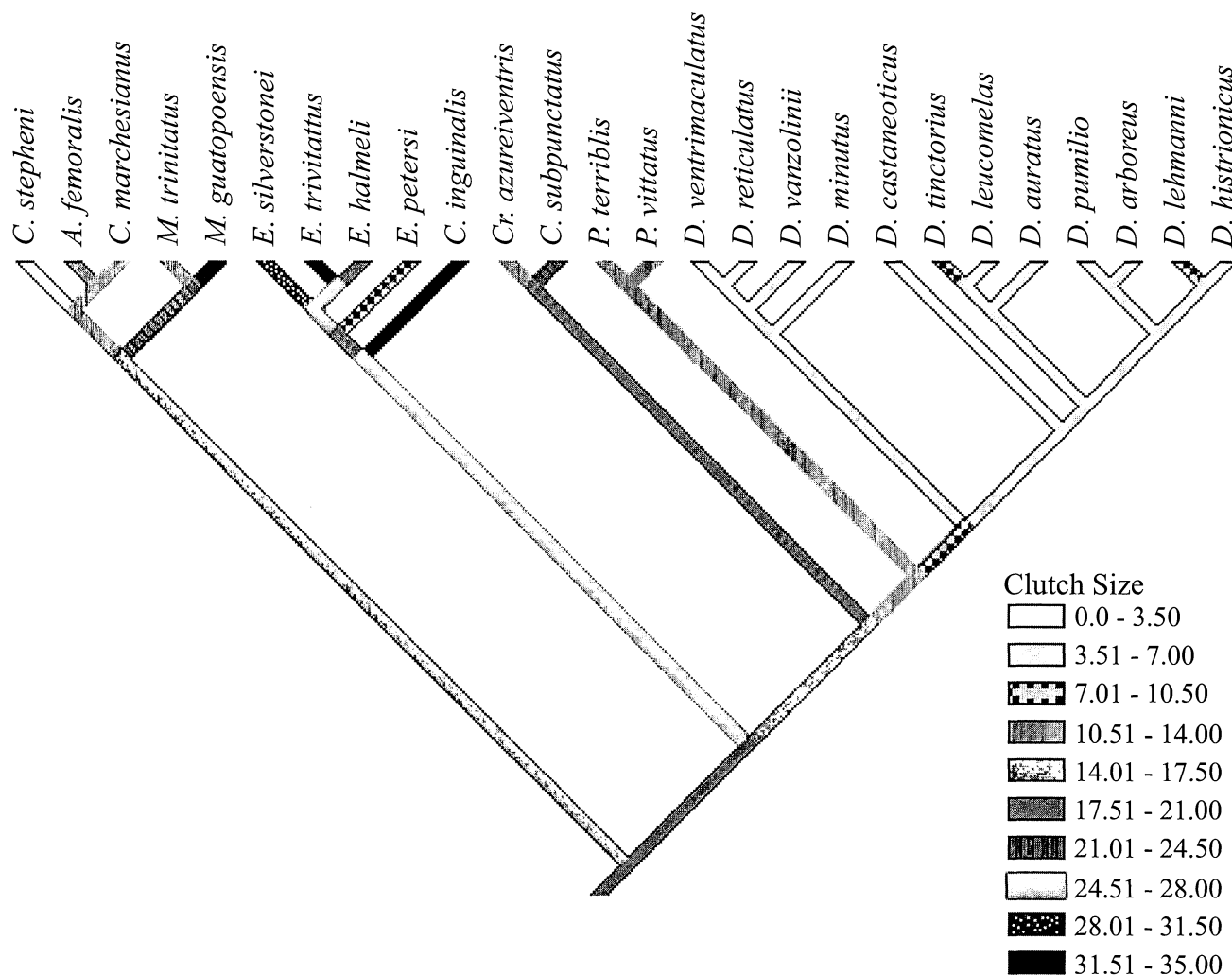

Fig. 5. The most parsimonious reconstruction (using squared change parsimony) of clutch size on the phylogenetic tree of dendrobatid frogs. Species for which data was not available have been trimmed from the tree. Tree construction and abbreviations as in Fig. 1.

care (Fig. 6b, male care: N = 18, X = 12.79, SE = 0.57; female care: N = 3, X = 9.67, SE = 0.83, Mann-Whitney U = 4.00, P = 0.021). Figure 7 and 8 map egg and tadpole size, respectively, on the phylogenetic tree of dendrobatids.

Why does this difference exist? Two factors seem likely to be important. First, in species with female parental care, females feed the tadpoles regularly over the course of the tadpole stage (Weygoldt, 1987). Hence, females do not have to provide a large amount of yolk to eggs in order to minimize the offspring's risk of starvation. In species where trophic eggs are not provided, extra yolk may be critical in allowing tadpoles to avoid starvation early in their development in small, nutrient poor phytotelmata. Second, tadpoles placed together in small phytotelmata may face high a high risk of cannibalism from larger conspecifics (Summers, 1990; Caldwell and Araújo, 1998). Species with female care generally deposit only a single tadpole in a pool, so that the risks of cannibalism are reduced (Brust, 1990). In species where cannibalism is common, it may pay females to produce relatively large offspring, to decrease their risk of being eaten (and increase their chances of being cannibals).

The first hypothesis predicts only that species with male

parental care will have larger egg and tadpole size than species with female care, which is the case (see above). The second hypothesis further predicts that species in which cannibalism is frequent will tend to have larger eggs and tadpoles, even within the species with male parental care. In general, species with male care that utilize phytotelmata seem to have the highest levels of cannibalism (Zimmermann and Zimmermann, 1984; Weygoldt, 1987; Summers, 1990, 1999; Summers and Amos, 1997; Caldwell and Araújo, 1998). Hence, the hypothesis predicts that among species with male care, species that breed in phytotelmata should have larger egg and tadpole size.

For egg size, there is a trend in the direction predicted by the second hypothesis, but it is not significant (Fig. 6a, phytotelmata: N = 5, X = 2.76, SE = 0.40; stream/pool: N = 15, X = 2.36, SE = 0.16; Mann-Whitney U = 25.5, P = 0.30). However, there is a significant difference in size for Gosner Stage 25 tadpoles (Fig. 6b, phytotelmata: N = 5, X = 15.43, SE = 0.99; stream/pool: N = 11, X = 11.67, SE = 0.53; Mann-Whitney U = 5.50, P = 0.013). This result is not confounded by body size, because there was no significant difference in body size between phytotelmata-breeders and stream/ pool breeders with male care. Given the relative inaccuracy of frog egg measurements (Crump, 1974), it is likely that the difference in tadpole size reflects a real difference in investment between phytotelmata and stream and terrestrial pool-breeding dendrobatids with male care.

Earlier in this paper we argued that inter-specific predation may have driven the transition from terrestrial pool-breeding to phytotelmata-breeding. It may seem contradictory to now argue that cannibalism imposes stronger selection on phytotelmata-breeding dendrobatids, favoring larger egg size. However, the argument concerning the effect of inter-specific predation focused on the ancestral period of the transition, at which point cannibalism would have been non-existent. The fact that cannibalism may now create stronger selection on egg and clutch size than inter-specific predation is not relevant to the former argument. Furthermore, there is a key difference between inter-specific predation and intra-specific predation (cannibalism), in that for most inter-specific predators (such as damselfly larvae), small differences in the size of the tadpole at deposition are not likely to save the tadpole from predation. In contrast, small differences in the size of the tadpole at deposition may well save a tadpole from being cannibalized by another tadpole that is only slightly larger.

In summary, egg, tadpole and clutch size seem to have been affected by the transition to phytotelmata-breeding. Egg and tadpole size have declined in phytotelmata-breeding species with female care, but tadpole size has increased in phytotelmata-breeding species with male care, possibly because of high levels of cannibalism. Clutch size has declined, possibly in response to the intensive effort required to locate suitable phytotelmata and transport each offspring to separate phytotelmata. Whether smaller clutch size reflects a lower overall investment, or simply a different distribution of
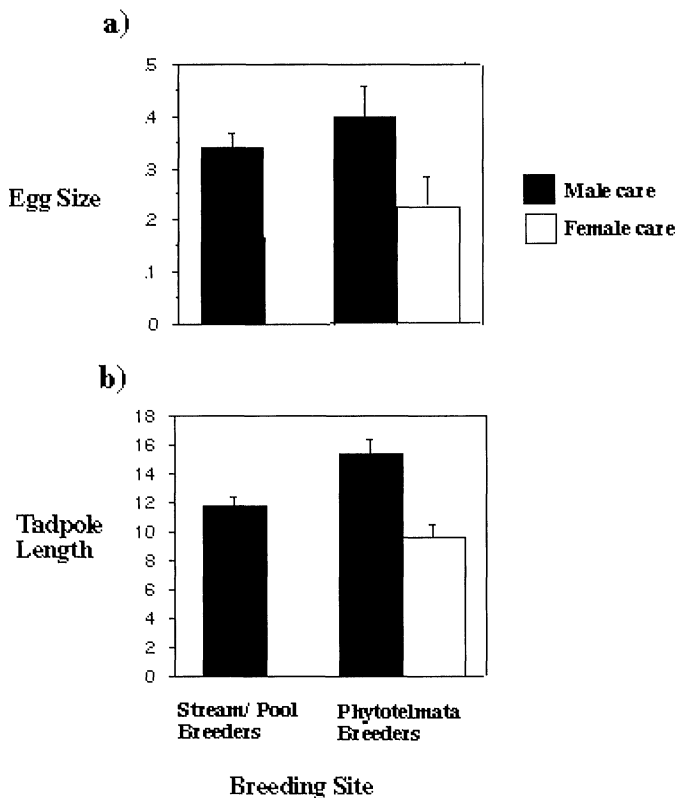

Fig. 6. a) Bar chart showing average egg size in stream and terrestrial pool-breeding dendrobatids, and phytotelmata-breeding dendrobatids, divided up by parental care type (male and female). b) Bar chart showing average tadpole length in stream and terrestrial pool-breeding dendrobatids, and phytotelmata-breeding dendrobatids, divided up by parental care type (male and female). Error bars show one standard error for each chart.

Fig. 7. The most parsimonious reconstruction (using squared change parsimony) of egg size on the phylogenetic tree of dendrobatid frogs. Species for which data was not available have been trimmed from the tree. Tree construction and abbreviations as in Fig. 1.
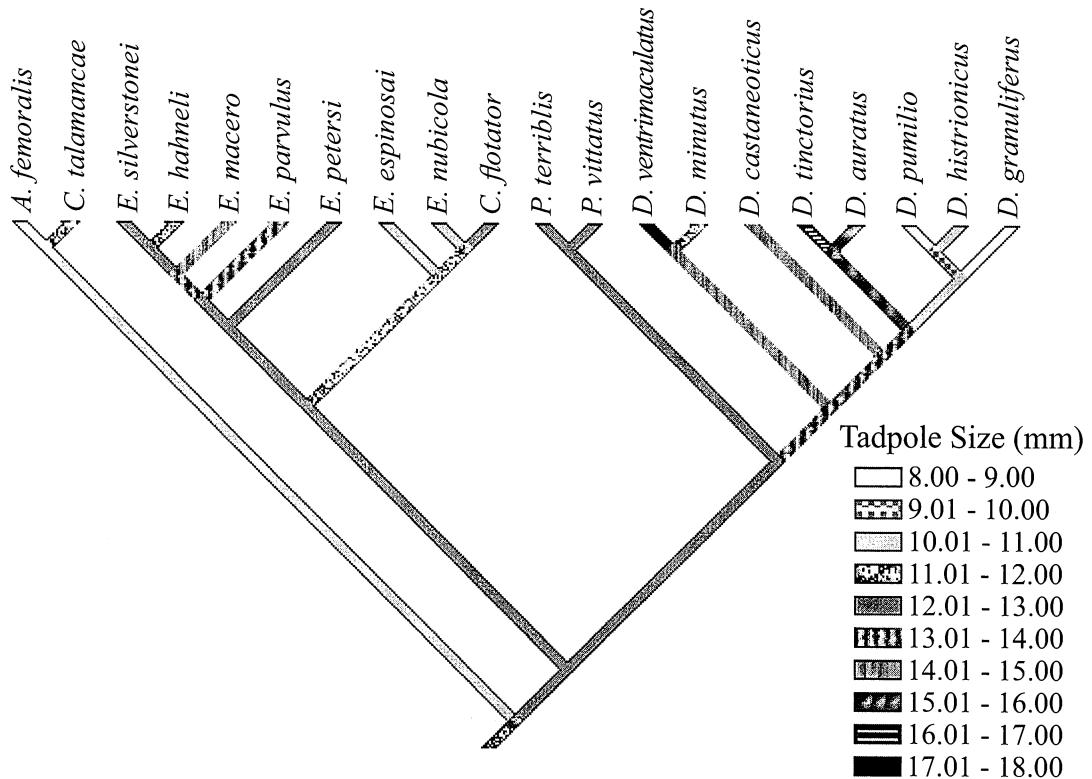

Fig. 8. The most parsimonious reconstruction (using squared change parsimony) of total tadpole length (stage 25 tadpoles) on the phylogenetic tree of dendrobatid frogs. Species for which data was not available have been trimmed from the tree. Tree construction and abbreviations as in Fig. 1.

clutches over time, is not known. Resolving this question will require more complete data on the reproductive schedules of dendrobatids in the field, information that is currently available for very few species.

Larval Ecology, Population Density, and Community Structure. As mentioned above, inter-specific competition among larvae appears to be rare in phytotelmata-breeding dendrobatids. It is not clear why this is the case, although specialization on different types of phytotelmata is a possibility. If such specialization occurs, then the availability of different types of phytotelmata could influence the abundance of particular species. Whether or not the availability of any type of phytotelmata affects population density in dendrobatids has rarely been investigated. Donnelly (1989*a,b*) established experimental and control plots in cacao groves at the La Selva Biological Station in Costa Rica, and manipulated the abundance of leaf litter and bromeliads in a two-factor factorial design. This experiment demonstrated a positive effect of phytotelmata (bromeliad) abundance on the population densities of both males and females in this population. However, this effect was due to increased immigration and decreased emigration of adults, rather than enhanced recruitment.

The degree to which tadpole growth and survival affects adult dendrobatid abundance and community structure has not been investigated. Here we present data that is consistent with a role for larval ecology on the relative abundance of two species of poison frogs that co-occur at the Nusagandi Biological Reserve in San Blas Province, Panama. Casual observations at this reserve reveal that *D. minutus* is very abundant. In contrast, *D. auratus* is rarely seen and hence appears uncommon. In order to confirm this initial impression, Summers (2002) used visual encounter surveys (Heyer *et al.*, 1994) to assess the relative abundance of leaf litter anurans on three different plots in the reserve. These surveys revealed that *D. minutus* is substantially more abundant than any other leaf litter species occurring in the area. The relative abundance of *D. minutus* was 0.36, compared to a second highest relative abundance of 0.14 for *Colostethus nubicola* (Summers, 2002). In contrast, *D. auratus* was present, but sufficiently uncommon that it was not detected during the surveys.

An obvious question arises as to why *D. minutus* is so much more common than *D. auratus*, particularly given their close phylogenetic relationship and similar ecological niche. Many things can influence relative abundance, but predation and diet are two of the most commonly invoked factors. Predation is unlikely to explain the difference in abundance. *Dendrobates auratus* is highly toxic, whereas *D. minutus* is relatively non-toxic (Daly *et al.*, 1987). Diet also seems unlikely to play a major role. Toft (1980) investigated the diet of these species (among others) in an area very close to the Nusagandi Reserve. Her research demonstrated that these two species have similar diets, and that their prey occur in abundance.

Another possibility is that larval ecology has an important influence on adult population densities. Larval interactions

between these species involving competition or predation could certainly influence adult population size. Differential availability of phytotelmata suitable for each species could also affect recruitment. Surveys carried out to locate phytotelmata for ecological experiments suggested that small bromeliads were substantially more abundant in the area than larger phytotelmata such as treeholes. We investigated the potential effects of larval interactions and pool availability with experimental manipulations (see Appendix 1).

Tadpoles of the two species collected during this study differed significantly in size: *D. auratus* tadpoles averaged 15.9 mm in total length, compared to 12.6 mm for *D. minutus* tadpoles at the same stage (stage 25). This is consistent with differences between the species in adult body size: adult *D. auratus* averaged 32.3 mm SVL, whereas adult *D. minutus* averaged 13.1 mm.

Three experimental treatments relevant to competition and predation between *D. auratus* and *D. minutus* tadpoles were carried out. Tadpoles were matched for size to minimize size and weight discrepancies between the different species. There was no significant size difference between the tadpoles at the start of the experiment. In spite of this size matching, in each case the *D. auratus* tadpole cannibalized the *D. minutus* tadpole within a week.

In order to evaluate whether *D. minutus* tadpoles were better able to survive and grow in small phytotelmata than *D. auratus*, experiments on the growth rates of single *D. minutus* and single *D. auratus* in small phytotelmata were carried out. *Dendrobates minutus* tadpoles were placed in smaller phytotelmata than the *D. auratus* tadpoles, to make the test conservative. There were significant differences between the growth rates of *D. auratus* and *D. minutus* for SVL (Fig. 9a; t-test: *D. minutus*: N = 10, X = 0.49; *D. auratus*: N = 4, X = -0.73; t = -3.22, P = 0.007) and head width (Fig. 9b; *D. minutus*: N = 10, X = 0.69; *D. auratus*: N = 4, X = -0.26; t = -2.39, P = 0.034), but not for total length. These results suggest that *D. minutus* tadpoles are able to grow in the readily available small phytotelmata, whereas *D. auratus* tadpoles are not. Since the *D. auratus* tadpoles shrank instead of growing in the small pools, it is unlikely that they would survive.

In summary, *D. auratus* tadpoles predate *D. minutus* tadpoles when they occur in the same phytotelmata. Nevertheless, *D. auratus* do not utilize the small phytotelmata used by *D. minutus*. This is probably because there are insufficient nutrients available for *D. auratus* tadpoles to grow and survive in those pools. This may have a significant effect on the abundance of adult *D. minutus* relative to *D. auratus* at this site.

Conclusions. We have explored the evolutionary ecology of phytotelmata-breeding by dendrobatids from many different angles. It should be apparent that many of the factors we have discussed, such as pool size, predation, competition, cannibalism, nutrient availability, patterns of parental care, mating strategies, reproductive parasitism, egg and clutch size, population density and community structure are intimately

connected through a complex web of interactions. As a visual summary, we illustrate some of these connections in Fig 10, which shows interactions between the factors we have discussed.

The ecological factors driving the transition from terrestrial pool-breeding to phytotelmata-breeding remain obscure, but progress in understanding what factors were likely to have been important can be made by investigating the effects of selected factors on extant species. The presence of a relatively contiguous range of reproductive mode within the extant Dendrobatidae allows for experimental testing of larval growth and survival, and adult habitat choice, in species utilizing breeding strategies that span the transition between terrestrial pools and phytotelmata. We have carried out preliminary research that is relevant to this issue. Experiments transplanting tadpoles of terrestrial-pool breeders to phytotelmata to investigate growth and survival effects may also prove useful.

The synthesis of multiple avenues of research will allow us to better evaluate particular hypotheses concerning the transition from terrestrial pool to phytotelmata-breeding. For example, if we find that predation by invertebrate predators is the most important factor influencing larval survival and adult habitat choice in dendrobatids that currently utilize terrestrial

pools, and if transplantation experiments reveal a pronounced survival advantage to larvae in phytotelmata, these results would support the hypothesis that predation was an important factor driving the transition to phytotelmata-breeding.

Many promising lines of ecological inquiry remain unexplored, and greater systematic resolution of the group will continue to add context to future work. Ultimately, the reproductive use of phytotelmata has bearing on most elements of the biology of the family Dendrobatidae. As such, an understanding of the evolution of this trait, and the ecological forces that drove the transition from the forest floor to arboreal phytotelmata, may be our most useful key to gain insight into this fascinating group.
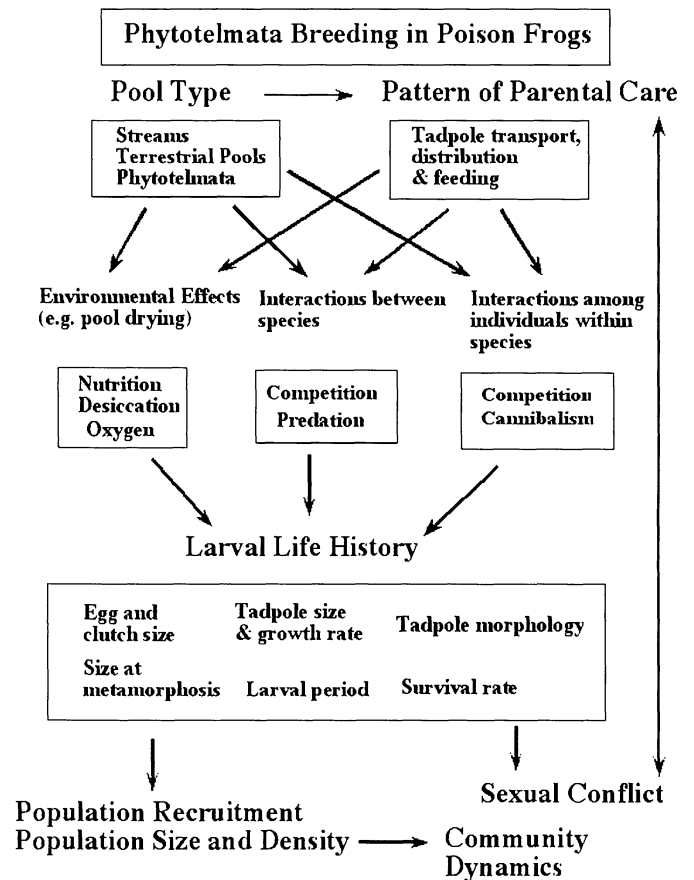
Fig. 10. Summary of connections between factors discussed in this review. The type of pool used for tadpole deposition affects the nature of parental care provided in particular species. In turn, pool type and the nature of parental care combine to influence the environment experienced by larvae, including abiotic aspects (e.g. probability of desiccation) and biotic aspects (e.g., probability of predation). These environmental factors strongly affect the evolution of larval life history strategies, and larval survival rates. In turn, larval survival can affect recruitment, adult population size and density, and community structure. Tradeoffs between the mating and parental care strategies of males can affect larval growth and survival, generating sexual conflict and potentially influencing the evolution of parental care patterns.
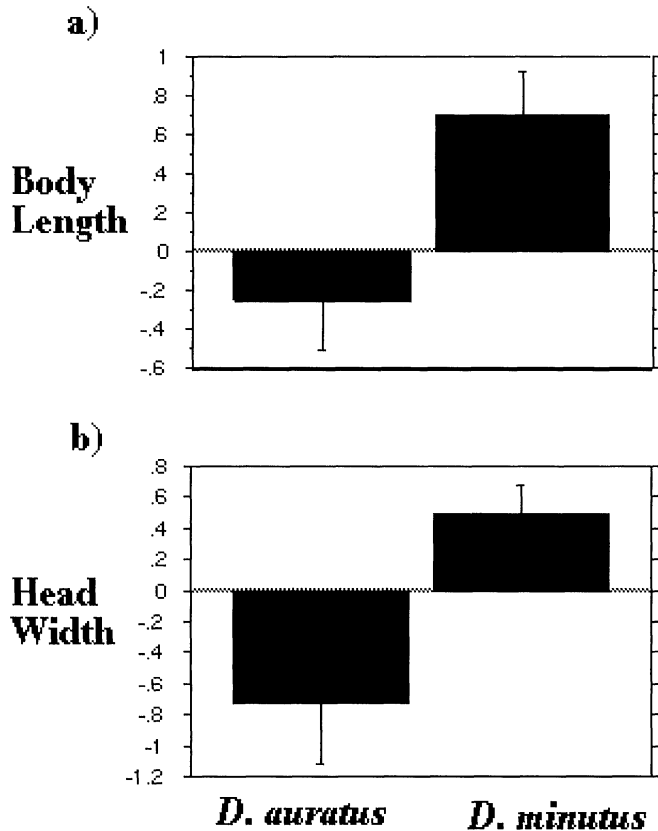

Fig. 9. a) Changes in body length for *Dendrobates minutus* and *D. auratus* tadpoles in phytotelmata experiments. b) Changes in head width for *D. minutus* and *D. auratus* tadpoles in phytotelmata experiments.

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Appendix 1: Field Methods

Dendrobates ventrimaculatus

Dendrobates ventrimaculatus was studied during June through August, 1997, in lowland rainforest near Pompeya, Ecuador. This species utilizes *Heliconia stricta* axils for both egg and tadpole deposition. Several hundred axil pools were monitored for several months (Summers, 1999). A 40 x 85 m grid was established in an area of high *Heliconia* density, marked with plastic flagging. Pools that contained eggs or tadpoles were monitored every other day for two months. A total of 241 breeding pools (containing eggs, embryos, tadpoles, or adults) were marked and monitored. A qualitative estimate of initial pool size (based on perceived similarity to pools for which volume had been measured by removal of all water) was made for each pool: Small (less than 20 ml), Medium/ Small (20-50 ml), Medium (50-100 ml), Medium/ Large (150-200 ml), Large (>200 ml). The final volume of 43 pools from which tadpoles were collected was measured by removing the water with a pipet. Pools were assigned to two final volume categories for an analysis of tadpole morphology: normal (20 ml or above), and low (less than 20 ml).

Tadpoles in pools with very low water levels (less than 20 ml) were collected approximately two weeks after the low water levels had been detected. Hence, tadpoles in these pools had several weeks to respond developmentally to pool drying. Tadpoles were also collected from pools that were not dry, for comparative purposes. Tadpoles were transported to the village of Limoncocha, where they were weighed on an electronic scale (Ohaus Model 300), measured (SVL and total length) and staged (Gosner, 1960). A total of 61 tadpoles were collected. Almost all (60) were weighed and staged. Most tadpoles were measured for SVL (58) and total length (52). Some were not measured due to damage, particularly to the tail. For the purpose of analysis, tadpoles were divided into two stage categories: Early (from stage 25 to 34), and Late (from stage 35 to 45).

Dendrobates minutus and *Dendrobates auratus*

Dendrobates minutus and *D. auratus* were studied at the Nusagandi Biological Reserve, in the Comarca of San Blas, Republic of Panama. Observations and experiments were carried out in three areas along the Ina Igar trail, in primary moist tropical forest.

Methods used for species abundance surveys were described by Summers (2002). Tadpole experiments were carried out in bromeliads collected from the field. Bromeliads were emptied of all detritus, tadpoles and insect larvae, filled with a small amount of detritus and water, and planted in plastic cups. Experiments were carried out on a sheltered porch at the Nusagandi Field Station and in fenced enclosures in the field. *Dendrobates minutus* tadpoles were collected from bromeliads in the field. *Dendrobates auratus* tadpoles were not found in bromeliads, but were collected by placing jars of water near areas where *D. auratus* adults were seen. Tadpoles were staged using Gosner's (1960) staging tables. Tadpoles were weighed on an electronic balance (Ohaus Model 300), and three morphological features were measured: total length, body length, and body width. The number of replicates per experiment was kept low to minimize the pain and trauma due to cannibalism or starvation. Statistical analyses were performed with Statview (Abacus, 1996).

Genus	Species	Avg. Egg	Avg. Clutch	Avg. Tad	Avg. SVL	Avg. F	Max. F	Breeding Site	PC
Allobates	*femoralis*	1.80	17.50			27.00		Stream/ Pool	Male
Allobates	*femoralis*	2.00	22.70	7.40		26.00	29.00	Stream/ Pool	Male
Allobates	*femoralis*			10.50	26.60	27.30	33.50	Stream/ Pool	Male
Allobates	*zaparo*				27.90	28.00	30.50	Stream/ Pool	Male
Aromobates	*nocturnus*			68.10	53.02	57.49	61.60	Stream	
Colostethus	*bromelicola*	1.70	4.00	14.50	16.03	17.35	17.20	Small Phyto	
Colostethus	*caerulodactylus*	1.18	19.20		15.80	16.10	16.80	Stream/ Pool	Male
Colostethus	*degranvillei*				18.26	19.67		Direct Devel.	
Colostethus	*flotator*			12.50	16.10	16.60	18.00	Stream/ Pool	Male
Colostethus	*guatopoensis*	3.00	34.00	16.00	35.00	37.00	43.50	Stream	Male
Colostethus	*inguinalis*		35.00		26.00	27.00	31.00	Stream	Female
Colostethus	*marchesianus*	2.00	15.50		16.50	17.00	18.00	Stream/ Pool	Male
Colostethus	*marchesianus*	1.50	14.00		16.50	17.00	18.00	Stream/ Pool	Male
Colostethus	*marchesianus*	1.00	9.20					Stream/ Pool	Male
Colostethus	*nubicola*			11.00	18.00	18.60	23.00	Stream/ Pool	Male
Colostethus	*palmatus*				32.15	33.57		Stream/ Pool	Male
Colostethus	*pratti*				19.35	20.03		Stream/ Pool	Female
Colostethus	*sauli*	1.50	11.00		24.25	24.01	28.00	Stream/ Pool	Male
Colostethus	*stepheni*	2.00	3.80				18.00	Direct Devel.	Male
Colostethus	*subpunctatus*		21.90		20.55	22.44		Stream/ Pool	Male
Colostethus	*talamancae*			12.00	21.75	23.90	25.00	Stream/ Pool	Male
Colostethus	*trilineatus*				16.77	17.09	19.30	Stream/ Pool	Male
Colostethus	*trinitatus*	3.50	12.00		24.35	25.60	29.00	Stream/ Pool	Male
Colostethus	*vertebralis*	3.00	8.50	11.70	17.47	18.50	20.20	Stream/ Pool	Male
Dendrobates	*arboreus*		5.00		21.00	21.26	22.20	Small Phyto	Female
Dendrobates	*auratus*	2.35	4.31	15.95	32.80	33.90	42.00	Large Phyto	Male
Dendrobates	*biolat*				15.25	16.00	17.00	Small Phyto	Bipar
Dendrobates	*castaneoticus*		2.40	14.94	20.60	21.90	22.70	Large Phyto	Male
Dendrobates	*fantasticus*				19.50	20.50	23.00	Small Phyto	Male
Dendrobates	*granuliferus*	2.16	4.54	10.50	20.70	20.80	22.00	Small Phyto	Female
Dendrobates	*histrionicus*	1.50	3.50	10.50	33.20	33.10	38.00	Small Phyto	Female
Dendrobates	*lamasi*				18.75	20.50	21.00	Small Phyto	
Dendrobates	*lehmanni*	1.50	9.50			32.80		Small Phyto	Female
Dendrobates	*leucomelas*	3.93	7.00		33.80	34.80	37.50	Large Phyto	Male
Dendrobates	*minutus*	3.50	2.00	12.00	13.35	13.50		Small Phyto	Male
Dendrobates	*mysteriosus*				27.00	27.00	29.30	Small Phyto	Male
Dendrobates	*pumilio*	1.10	6.00	8.00		20.70		Small Phyto	Female
Dendrobates	*reticulatus*	2.00	2.50		14.75	15.50	17.00	Small Phyto	Male
Dendrobates	*tinctorius*			16.25	27.20	46.50	50.00	Large Phyto	Male
Dendrobates	*vanzolinii*		5.20		17.80	18.60	19.90	Small Phyto	Bipar
Dendrobates	*variabilis*				17.43		19.00	Small Phyto	Male
Dendrobates	*ventrimaculatus*	2.00	2.93	18.00		15.42		Small Phyto	Male
Dendrobates	*ventrimaculatus*				16.00	16.50	18.00	Small Phyto	Male
Dendrobates	*ventrimaculatus*				17.10	17.30	21.50	Small Phyto	Male
Epipedobates	*anthonyi*				19.20	20.00	21.00	Stream/ Pool	Male
Epipedobates	*azureiventris*		15.00		22.85	24.70	28.00	Stream/ Pool	Male
Epipedobates	*bassleri*		26.00		37.30	37.50	42.00	Stream/ Pool	Male

Avg. Egg = average egg diameter (mm), Avg. Clutch = average clutch size, Avg. Tad = average tadpole length (mm), Avg. SVL = average adult snout-vent length (mm), Avg. F = average female snout-vent length (mm), Max. F = maximum female snout-vent length (mm), PC = parental care type, Direct Develop. = Direct Development, Small/Large Phyto. = Small or Large Phytotelm

Appendix 2: Egg and Clutch Size Data

Genus	Species	Avg. Egg	Avg. Clutch	Avg. Tad	Avg. SVL	Avg. F	Max. F	Breeding Site	PC
Epipedobates	*bolivianus*				24.80	25.80	26.00	Stream/ Pool	Male
Epipedobates	*boulengeri*				17.70	18.50	21.00	Stream/ Pool	Male
Epipedobates	*espinosai*			11.00	16.90			Stream/ Pool	Male
Epipedobates	*hahneli*	2.30	22.00		19.75	20.50	22.00	Stream/ Pool	Male
Epipedobates	*hanheli*		18.00	11.50		24.40		Stream/ Pool	Male
Epipedobates	*macero*			14.10	27.20	28.59		Stream/ Pool	Male
Epipedobates	*parvulus*	3.00	8.90	13.20		21.10	23.00	Stream/ Pool	Male
Epipedobates	*parvulus*				20.50	21.20	24.00	Stream/ Pool	Male
Epipedobates	*petersi*	3.00	10.00			29.00		Stream/ Pool	Male
Epipedobates	*petersi*			12.50	26.60	28.00	31.00	Stream/ Pool	Male
Epipedobates	*pictus*	2.00	18.00			22.10	24.00	Stream/ Pool	Male
Epipedobates	*pictus*				23.80	24.40	31.50	Stream/ Pool	Male
Epipedobates	*pulchripectus*				24.50	26.00	27.00	Stream/ Pool	Male
Epipedobates	*silverstonei*	2.00	30.00	12.42	39.00	41.83	42.80	Stream/ Pool	Male
Epipedobates	*tricolor*		20.00		23.70	24.40	26.50	Stream/ Pool	Male
Epipedobates	*trivittatus*	3.00	38.00			45.00		Stream/ Pool	Male
Epipedobates	*trivittatus*		27.00	20.50	39.80	43.60	49.50	Stream/ Pool	Male
Mannophryne	*collaris*				23.82	25.43		Stream	Male
Mannophryne	*herminae*		12.70	13.60	21.98	22.99		Stream	Male
Phyllobates	*aurotaenia*			10.48	27.70	30.40	34.00		Male
Phyllobates	*bicolor*				38.20	39.80	42.00	Stream/ Pool	Male
Phyllobates	*lugubris*			10.39	20.50	22.20	23.50		Male
Phyllobates	*terriblis*	2.50	14.00	11.65	42.00	43.23	47.00	Stream/ Pool	Male
Phyllobates	*vittatus*	2.50	14.00	12.70	25.70	27.70	29.00	Stream/Pool/Phyto	Male

Avg. Egg = average egg diameter (mm), Avg. Clutch = average clutch size, Avg. Tad = average tadpole length (mm), Avg. SVL = average adult snout-vent length (mm), Avg. F = average female snout-vent length (mm), Max. F = maximum female snout-vent length (mm), PC = parental care type, Direct Develop. = Direct Development, Small/Large Phyto. = Small or Large Phytotelm

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