

# Leaf Litter

The Magazine of Tree Walkers International and Amphibian Conservation



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The Magazine of Tree Walkers International

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## MISSION STATEMENT

Tree Walkers International supports the protection, conservation, and restoration of wild amphibian populations through hands-on action both locally and internationally.

We foster personal relationships between people and nature by providing opportunities for citizens of all ages to become directly involved in global amphibian conservation.

Through this involvement, our volunteers become part of a growing and passionate advocacy for the protection and restoration of wild amphibian populations and the environment on which they depend.



## Director's Welcome



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## A 500-Year Plan

What if we were to plan not for just the next year, but were looking at amphibian conservation for the next 500 years? If we look at the natural state of the world of 500 years ago and compare it with today, we have reason to be both optimistic and pessimistic.

It is a fascinating, exciting time for discovery in the global amphibian community. The Internet makes it possible to see the striking image of a red-headed fantasticus while next-day air delivery makes delivery of wonderful animals to our doorstep from across the world a reality. However, this interconnectedness must not cloud the real and significant challenges we face today nor give us a false sense of accomplishment or obscure the fact that, in our own backyards, amphibian habitat is often lost to development and that many of our native species are under a variety of threats.

It is our responsibility as conservationists from throughout the commercial, private, and scientific sectors to come together and, one year at a time, work toward a long term goal that meshes the interests of our diverse membership and brings us together to protect amphibians and conserve their habitats not just for now, but for millennia.

I am confident that Tree Walkers International stands at the vanguard of a holistic conservation approach that does not care if you are a zoo keeper or basement frog keeper, but instead leverages a common love for amphibians and their natural environment. While I cannot guarantee that we will be around as an organization in 2507, I look forward to working with all of you for as long as we are needed.

Sincerely,

**Marcos Osorno**  
Executive Director

# An Attempt to Breed *Atelopus flavescens* by Artificial Means

by Peter Mudde

**I**n the summer of 1988 I received a small group of *Atelopus flavescens* through a hobbyist friend. There were four males and a single female in the group; the entire group was from the region of Montagne Matouri in French Guyana. The group was housed in a vivarium with a floor area of 60 by 60 cm (@ 24 x 24 inches) with a sloping glass cover. The vivarium was planted with several epiphytes, which were grown in Xaxim™ (tree fern root) pots standing on stones. The available terrestrial area was restricted to the pots, the parts of stones extending out of the water and the plants that grew throughout the vivarium.

I used rainwater to fill the vivarium. This was sprayed through the vivarium by means of a small pump which fed two tubes one on each side of the vivarium. This way it was always very humid inside the vivarium, somewhere between 80 and 99% relative humidity. The tank was illuminated by two 15 watt fluorescent tubes, and the heat from the transformers of these fixtures was used to heat the tank. This way inside temperatures varied from 20 deg C in the night to 28 deg C in the late afternoon on normal days. Occasionally in the summer the temperature would exceed 28 C and during the winter on at least two occasions the temperature dropped to below 14 C. Neither of these extremes appeared to affect the frogs.



The frogs were fed ad libitum with fruit flies, which were dusted with minerals and vitamins of all kinds. I used a different brand every time, to get as much variation as possible (or, because I could not get the brand I had before. To be honest, the variation story is wisdom in hindsight.) During the summer the frogs were occasionally fed with a variety of other insects from meadow-sweepings. I also collected rotting leaves from beneath trees in our neighbourhood, which were placed in the vivarium in the hope that there would be small arthropods in the leaves, which the frogs could then consume.

After a year, the males became interested in the female and one crawled on her back in amplexus. (Editor's note: in captive populations of *Atelopus varius*, *A. spumarius* ssp. and *A. zeteki*, males will enter into amplexus with any female encountered regard-

less if the females is ready to breed or not. In the wild, males tend to only encounter females when the females descend to the streams to oviposit.) I had heard several horror stories about this, as the males tend to stay in amplexus until the eggs are deposited and females are very reluctant to deposit eggs. In other words, the male could stay on the back of the female forever or to be precise, until either the male or the female dies. (Editor's note: In captive populations of *Atelopus zeteki*, lesions have developed on the feet of females in amplexus that result in potentially lethal bacterial infections. The lesions are apparently due to the female either trying to get the male loose and/or carrying the weight of the male). I tried to pull the male off the back of the female but to no avail—within an hour another male had mounted the female. Another hobbyist told me he once broke an arm of a male while trying to get him off, so I

left them alone and hoped for the best. For three months the male remained on the back of the female.

When I observed the pair, I could occasionally see the belly of the female where eggs were developing. This made me hopeful, but even after a month's worth of waiting there was no progress. The male became thinner and thinner and I started to fear for the health of the male, as well as the possibility that the female might reabsorb the eggs. I decided to try to induce spawning by injecting the female with hormones. I managed to get some human gonadotropine (Pregnyl™) and a very small injection needle. (Editor's note: gonadotropine used for salmon are more effective for inducing oviposition in amphibians than mammalian gonadotropins.) I am lucky enough to be married to someone whose profession is to inject substances into people, so I had no fear regarding that.

In an article on artificial breeding of *Litoria caerulea* (Meyer & Schneider 1988. Das Timing von Amphibien mit gonadotropen Hormonen am Beispiel von *Litoria caerulea* (White 1970) Herpetofauna (in German) 10(55) 13-16) I found a dosage used for *Litoria caerulea*, 5000 IE/animal. I calculated that my frogs were about one-hundredth of the weight (female 4 g, male 2 g,) of the *L. caerulea* and, because in *L. caerulea* a dose of 400IE did make the frogs spawn, I decide to be on the safe side and inject 50IE in the female and half that dose in the male. The injections were given under the skin of the back with as fine a needle as possible. For the frogs this is quite invasive as the needles cause relatively large holes that do not heal immediately. I refrained from injecting into a leg as I thought any movement might press the injected fluid out. (Editor's note: depending on the quantity, it is possible to give injections into the legs without issue.

Also, the injection of hormones can be injected into the coelomic cavity.) The first injections produced no results. A week later, a second attempt was made with a tenfold dose. Both the male and female were injected (again under the skin of the back) with 500 IE of gonadotropin. This produced the desired results: the next morning there were white eggs all over (and under) the submerged parts of the tank (stones, twigs...everywhere). I estimated a total of 250-300 eggs. (Editor's note: In *A. zeteki* and *A. varius*, oviposition occurs under stones and other debris. In captivity this can be supplied through the use of deep water and upside down clay pots with access holes provided for the frogs.)

The eggs were removed from the vivarium to previously prepared small aquaria I had ready, each with an air stone powered by an air pump to provide constantly moving water (which was demineralised). After four days a single egg showed signs of development. In other eggs a small change in size indicated development. After nine days the first tadpole left the egg-capsule and a week later the last tadpole hatched, resulting in a total of fifteen larvae.

The water temperature in the tank was maintained between 21 and 23 C° (Editor's note: in *A. zeteki* and *A. varius*, keeping the tadpoles below 21.1 C results in the tadpoles not feeding well and wasting away). The larvae were fed with Liquifyr, algae (grown in an aquarium on a stone which was then placed in the tank), Spirulina powder and yeast. The tadpoles seemed to primarily consume the debris that gathered in the corners of the aquaria where the movement of the water was the least. Yeast resulted in no success and the larvae avoided it as much as possible. (Editor's note: Tadpoles of *A. zeteki* and *A. varius* can be reared on an artificial diet of

commercial fish foods that have been ground to a fine powder, mixed with water and offered as a paste smeared on rocks.) The water was changed and uneaten food was removed four times a week from one tank, once a week from another. In the end, the intervals in water changes didn't seem to be of much importance.

In three weeks time, a few larvae had grown to about twice the size of the eggs. A few larvae seemed to just disappear from within the tanks. After eight weeks I had nine left, three of which were slightly larger than the others. From that point on, growth ceased in all the larvae. Four weeks later all larvae were gone except one. It survived two more weeks before dying. It was stored on alcohol and studied. It had reached something between Gosner stage 26 and 30. As far as I could observe there were no differences from the larvae-description by Lescure. The last larvae is in the Zoological Museum of Amsterdam (now Leiden, I suppose)

This story is a bit like that of Mebbs (1980) in that his larvae also stopped growing around the same stage. The few successful results in breeding *Atelopus* I have seen were all single larvae reared in a relatively large volume of water. I think growth-inhibiting factors in the larvae's own excrement are the cause of the problem. These have been found to influence development in toads like *Bufo bufo*, and larvae used to a constant flow of clean water might be much more susceptible to such factors. (Editor's note: Tadpoles of *A. zeteki* and *A. varius* have been raised successfully communally in 208.2 liter aquariums.)

(Since writing this article, the author has made contact with someone who is going to have an opportunity to visit the breeding locale of *A. flavescens* in French Guyana. Therefore, this account is tentatively “to be continued...”)

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#### Works Cited

Amphibian Medicine and Captive Husbandry; 2001; eds Whitaker, Brent; Wright, Kevin; Krieger Press; Malabar

Karraker, Nancy E.; Richards, Corinne E.; Ross, Heidi L.; 2006; Reproductive ecology of *Atelopus zeteki* and comparisons to other members of the genus; Herpetological Review 37(3): 284-288

Starrett, Priscilla; 1967; Observations on the life history of frogs of the family Atelopodidae; Herpetologica 23(3): 195-204

Poole, V.; 2003; Studbook report to the AZA Amphibian TAG Panamanian Golden Frog *Atelopus zeteki*

Project Golden Frog; captive husbandry recommendations for the Panamanian Golden Frog *Atelopus zeteki*, 2001, AZA Publication, National Amphibian Conservation Center

### Open Call for Tadpoles

I am looking for tadpoles for a study on the effects of pH and water pollution on survival and development in tropical amphibians. This work will utilize the same methodology as my past work on the effects of temperature on *Dendrobates auratus* (Korbeck & McRobert, 2005). Any tropical species that produces large numbers of tadpoles that are easy to raise should work for the proposed study. If you have surplus tadpoles and wish to be involved in research (and possibly serve as an author on publications), please contact me:

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