

## Adventures with Amphibians

J.R. Downie

School of Life Sciences, Graham Kerr Building,  
University of Glasgow, Glasgow G12 8QQ

E-mail: roger.downie@glasgow.ac.uk

---

### INTRODUCTION

A retirement lecture gives an opportunity for looking back and reviewing, and attempting to give coherence to a career. I can think of scientists who set off early on a theme and pursued it doggedly throughout their careers. Mine hasn't been like that. I began as an avian embryologist, then got interested in the reproductive ecology of amphibians. Then, through involvement in student expeditions overseas, marine turtle life histories and conservation developed as a side interest. Along the line bioethics and evolution education became research themes too, so there is little coherence, but diversity of interests is not such a bad thing for a biologist.

I've chosen to concentrate here on amphibians, and have called my experiences 'Adventures', partly because amphibian work is often at night, and in the tropics, night work in swamps can lead to all sorts of unexpected happenings. 'Chance encounters might be a better title, because chance has played a major part in the research I've been able to do. My interest in amphibians grew out of teaching a course on reproductive biology, mainly in the vertebrates. In 1982, I got the chance to spend five months study leave in Trinidad. This was quite serendipitous. Robin Bruce, an ex-student who had been with us on an expedition to Iceland (1972) obtained his first lecturing post at the University of the West Indies in Trinidad. He reported that Trinidad and Tobago were good places to study frogs, and that his head of department had written the guide to them (Kenny, 1969). So my family and I went to Trinidad and I got hooked for life, having now visited the islands more than 20 times. In recent years, I've become involved in amphibian work in the UK too, but in this lecture, I'll concentrate on four themes from our Trinidad work.

### FOUR AMPHIBIAN ADVENTURES

#### *Foam-nesting frogs*

My first serious work in Trinidad in 1982 shows the influence of luck and chance. We arrived in the dry season with not many frogs around. Then there was some patchy rain, and puddles began forming on a piece of waste ground on the UWI campus: I passed these each day. After a heavy shower, I was surprised to find well developed tadpoles in the puddle as well as

floating foam nests. I was fairly sure there had been no tadpoles previously. A few dry days and the puddle dried up. Hard luck on these tadpoles and the foam nests, I thought, but then it rained again, and there were tadpoles again. What was going on? Next time it dried, I searched the damp mud at the low point and found, under dead leaves, an aggregation of tadpoles, wriggling about in a little heap of froth. A bit more searching, and in a burrow, under a stone was a white foam nest, containing early hatchling tadpoles. This was my first encounter with the whistling frog *Leptodactylus fuscus*, and it was a matter of luck because the ground staff tidied up the area a few years later and the frog populations disappeared. *L. fuscus* is a foam-nesting species, and often shares breeding pools with another foam nester, the tungara frog *Engystomops pustulosus*, which produces the floating foam.

An aside at this point: one of the real bugbears of working on tropical amphibians is unstable taxonomy. This is partly a consequence of colonial times with French, Dutch, Spanish and British natural historians giving different names to what turned out to be the same species, then having to sort out the mess. *L. fuscus* has had NINE names: *Rana fusca* (1799), *Rana typhonia*, *Rana sibilatrix*, *Leptodactylus typhoni*, *Cystignathus typhoni*, *Cystignathus fuscus*, *Leptodactylus sibilator*, *Leptodactylus sibilatrix*, *Leptodactylus fuscus* (1983). More recently, molecular phylogenetics have revolutionised our understanding of the evolutionary relationships of amphibians and unleashed a continuing spasm of taxonomic revision. Few of the frog species I first encountered in Trinidad in 1982 now have the same names. This is a curse, but also an opportunity: the unravelling of relationships has generated many opportunities to think again about the evolution of life histories and other adaptive features, as we'll see later.

When I started, the Trinidad tungara frog was *Eupemphix* then *Physalaemus*, now *Engystomops* and it is now in a separate family, the Leiuperidae, no longer a leptodactylid. Here are some of the things we found about these two frogs (Downie & Nicholls, 2004).

#### *Leptodactylus fuscus*

- Eggs are deposited in hidden burrows close to where temporary pools form. Burrows are closed by mud 'lids'. Most nests are deposited on dry nights, not after rain. We find them by prodding a spoon handle into the mud.
- Hatching occurs after 3 days. Tadpoles make a new kind of foam via oral secretions, and tadpoles can remain in this, without developing further, up to 4 weeks.
- If heavy rain falls, the tadpoles – already developed beyond hatching stage, enter the pool as the nest opens up – and are capable of eating newly deposited eggs of other species.

- The longer tadpoles stay in foam, the poorer their condition, and less able they are to grow successfully to metamorphosis.
- Burrow nests seem an excellent adaptation to an unpredictable environment but they do face a threat: a species of phorid fly, ‘frogflies’, can deposit their eggs in the foam and become maggots fast enough to destroy the frog embryos (this turned out to be a new species that we were able to describe).
- If the pond dries up, tadpoles can shelter under leaves or rocks on the damp mud surface and can survive several days – our original observation.

#### *Engystomops pustulosus*

- Floating foam nests are laid after rains. Hatching occurs after 2-3 days, with some larvae emerging late from the nest, possibly allowing development to a more advanced stage.
- Tadpoles have no ability to survive if the pond dries up.
- In competition with *L. fuscus* tadpoles, *E. pustulosus* tadpoles fare poorly – taking longer to reach metamorphosis and at a smaller size.

**Conclusion:** *L. fuscus* seems the superior competitor when pools are temporary and rainfall is unpredictable. Yet you see *E. pustulosus* everywhere: it seems especially well suited to human-related habitats like flooded tyre-ruts and must be a very effective colonizer of disturbed habitats. Perhaps the larger clutch size (about 100 for *L. fuscus*; 400 for *E. pustulosus*) is important here.

#### ***Phyllomedusa*: one of the charismatic poster frogs**

Here we have a piece of Glasgow Zoological history (Downie, 1997). The first generally cited paper on *Phyllomedusa* is by J.S. Budgett (1899) – a friend and colleague of John Graham Kerr’s before Kerr came to Glasgow. Budgett’s observations of *Phyllomedusa* were made on the Gran Chaco (Argentina-Paraguay) expedition where Kerr collected the lungfish embryos that were to be his main study. The second widely cited paper is by Wilfrid Agar (1910): Agar joined Graham Kerr in the Zoology Department in Glasgow and made observations on *Phyllomedusa* during another lungfish collecting trip in 1907-8 (not accompanied by Kerr). Agar eventually became Professor of Zoology in Melbourne, Australia from 1920. Both Budgett and Agar made important observations on *Phyllomedusa* reproduction, and we have recently been able to extend these.

The Phyllomedusinae are a sub-family of a major treefrog family, the Hylidae. There are (so far) 60 species of Phyllomedusine frogs, distributed throughout the neo-tropics, from Argentina through Central America and into Southern Mexico (Frost, 2011). There are two main genera, *Agalychnis* (including the widely-photographed *A. callidryas* – the

red-eyed treefrog) and *Phyllomedusa*. Phyllomedusines do not deposit their eggs in water. In *Agalychnis* eggs are deposited in clumps on open leaves overhanging water in wet rainforest. Since 1996, Karen Warkentin has published a large number of studies on *Agalychnis* based on her original finding that *Agalychnis* development shows considerable plasticity: egg clutches are heavily predated by snakes, but embryos can detect them and – within limits – hatch prematurely to escape the snakes, which tend not to swim after them. There is a classic trade off in operation here: embryos which develop fully on the leaf before hatching do better once they reach water than premature hatchlings: but if snakes attack, all embryos may be eaten, so premature hatching gives them a survival chance (Warkentin, 1999).

In comparison, *Phyllomedusa* incubation and hatching has been comparatively neglected, with few publications since Pyburn (1980). In Trinidad, we have *Phyllomedusa trinitatis* (which also occurs in northern Venezuela): in *Phyllomedusa*, eggs are deposited as an elongated clump on a leaf, but the adults use their limbs to fold the leaf around the clump, often managing to enclose the egg clump more or less completely – with an opening top and bottom. These openings are plugged by dense masses of jelly. Throughout the egg clutch are scattered large numbers of small round jelly capsules. The jelly capsules and plugs, and the eggs themselves (with thin jelly coats) are adhesive – so once the leaf has been folded over the clutch, it sticks in place. Generally, the leaf-nest overhangs a pool of water, so when the embryos hatch and emerge from the nest, they drop into the water below. But it seems not always possible for adults ready to reproduce to find a suitable leaf or leaves in a good place. They have two solutions. First, there may be better leaves a short distance away from the pool: once hatchlings emerge, they land on the ground, and are well capable of moving to water by flipping movements of their already powerful tails. Second, they can make rudimentary nests even from blades of grass overhanging a pool: we don’t think these are very successful, and it would be interesting to investigate what factors drive frogs to make this choice.

Agar (1910) suggested that the jelly capsules help hydrate the eggs, since he noticed that during incubation, eggs swell with fluid and jelly capsules shrink: the hydration role of jelly capsules has been confirmed (Pyburn, 1980). How the frog’s oviduct is able to make separate secretory releases (top and bottom jelly plugs, quite complex structurally; scattered capsules; a thin jelly coat round each egg), is not clear.

What we’ve found (work in preparation):

- Contrary to previous reports, eggs do develop in aquatic media, the better the later they enter water

and the better if the medium is a dilute balanced salt solution.

- The covering leaves do not need to be alive: incubation in cut leaf-nests is as successful as in live leaf-nests.
- Hatching of individual eggs can be stimulated by immersion in water, once they are hatching competent, or by contact with already hatched larvae, by a kind of chain reaction.
- Emergence from the nest does not occur until the lower jelly plug has been dissolved – probably as a result of enzymes released by larval hatching gland cells.
- Because of the time-lag between individual egg hatching and nest emergence, we do not think that premature hatching in response to predator attacks is relevant in *Phyllomedusa*. Rather, the leaf-fold nest and jelly plugs act as effective barriers to many kinds of predators.
- But there is a puzzle, yet to be resolved: how do *Phyllomedusa* eggs respire when they are apparently completely cut off from the air by leaf and jelly?

#### **The diversity of embryo-specific surface structures**

I've been fortunate for some years to have Mohsen Nokhbatolfoghahai working with me on the diversity of embryo and larval specific structures such as:

- Surface ciliation
- External gills
- Cement glands
- Hatching gland cells
- Tails

Scanning electron microscopy of these structures produces beautiful images (for example, Nokhbatolfoghahai & Downie, 2005). The example discussed here is that of the tails of direct-developing frogs, and luck is again important.

There are several lineages of anurans where a trend involving egg size increase and incubation on land has led to the suppression of the tadpole stage, and direct development to a juvenile frog. Generally, this involves the deletion from development of several larval specific structures, such as cement glands and external gills. But the tail is retained in modified form through the incubation stage, usually being resorbed just before hatching. We had included some observations on a Trinidad species *Eleutherodactylus urichi* in our paper on surface ciliation (Nokhbatolfoghahai *et al.*, 2005).

Then Nicola Mitchell (Western Australia) asked us to look at some direct-developing embryos of the turtle frog, and we elected to do this as a more detailed comparison with *Eleutherodactylus*. However, molecular phylogenetics had by then sub-divided the vast neo-tropical genus *Eleutherodactylus* (800+ species) into three main ancient sub-lineages and given

the whole group super-family status (Hedges *et al.*, 2008). Our *Eleutherodactylus* was no longer in that genus: now *Pristimantis*, whereas the only other member of the group whose embryos had been looked at in detail, the coqui frog of Puerto Rico, remained *Eleutherodactylus coqui*. This taxonomic revision made us look harder, and we discovered a very surprising feature (Nokhbatolfoghahai *et al.*, 2010). In most direct-developing frogs, the tail is retained, but as a respiratory organ with highly vascular skin. In the coqui frog and in the turtle frog, this involves some elongation of the tail fins to increase respiratory exchange surface area. But in *Pristimantis urichi*, we found that tail surface area expanded not by fin elongation, but by lateral expansion of skin, blood vessels and connective tissue. In coqui frogs and turtle frogs, the tail has well developed muscle, allowing the tail to move from side to side. In *Pristimantis*, the muscle is reduced, so that the tail has become a fixed respiratory organ, with its outer surface close to the inner wall of the vitelline membrane – in some ways reminiscent of the allantois in amniote embryos (though referees would not allow us to suggest that analogy!). Will this evolutionary innovation be found in other *Pristimantis*?

#### **Manno the stream frog**

I talked about our work on *Mannophryne trinitatis*, Trinidad's only dendrobatid (now aromobatid) in my 2005 Presidential Address (Downie, 2005), so I won't go over this in detail again. However, there is a footnote to the story. *M. trinitatis* males guard the eggs on land till they hatch. The male then carries the tadpoles on his back till he finds a suitable stream to deposit them into. Our work showed that suitable streams can be hard to find – since the males avoid leaving tadpoles in streams containing predators such as *Rivulus* fish. This is another case of good luck: for some time, I'd wondered where the males deposited their tadpoles, because we knew of many streams with large frog population but no tadpoles (but abundant *Rivulus*). Then one trip we chanced on a stream with hundreds of tadpoles (and no fish). Since each male only carries about 12 tadpoles, clearly frogs were coming to this stream from some distance, to avoid fish predation. Eventually, I realised that these males are on a dangerous quest and wrote it up as a children's story. We've now successfully told it – in the form of a play – to children's groups in Scotland and Trinidad. In my view, we need to develop stories about animals that can grab the attention of young people and help re-connect them with nature – if we are to halt the alarming declines in biodiversity we see everywhere – and in amphibians in particular.

To give the story 'human' interest, Manno the male stream frog meets a female called Trini, and really fancies her. Trini is older and a bit bossy and when Manno suggests that after he's found a good stream for their first batch of tadpoles, that they might get

together again “to get to know each other better” – Trini is pretty sharp with him.

I put in the idea of Manno and Trini becoming a faithful pair to add some human interest, and knowing that monogamy isn't supposed to happen in any frogs. However, a recent report shows that it does, and in a species of the same general type as *M. trinitatis*. In the mimic poison frog *Ranitomeya imitator*, males transport tadpoles on their backs to tiny bromeliad tanks. There is so little water and food per tank that tadpole growth depends on the female depositing trophic eggs to feed the tadpoles. The male stays on guard, and calls the female when more food is needed. Clearly, they care for the young together – and a possible hypothesis, yet to be tested, is that if the parents are successful in their different parental roles, it makes sense in terms of Darwinian fitness to remain together as a pair – for life (Kokko & Jennions, 2010).

### CONCLUSION

Most people are now aware of the serious threat to amphibian populations around the world. In my view, we need to do a lot more of the basic natural history work I've described, in order to understand better the lives of amphibians. If we don't, we are unlikely to devise sensible and effective conservation procedures.

### ACKNOWLEDGEMENTS

This paper is the edited text of the Presidential Address delivered to the Glasgow Natural History Society in October, 2010. The Address also acted as the 10<sup>th</sup> Blodwen Lloyd Binns lecture and as a lecture to mark my retirement after 40 years on the staff of the University of Glasgow. I'd like to thank the many undergraduate and postgraduate students who have contributed to the work described here. I'd also like to acknowledge the funders of this work, especially the Carnegie Trust for the Universities of Scotland, the University of Glasgow and the Blodwen Lloyd Binns bequest.

### REFERENCES

Agar, W.E. (1910). The nesting habits of the tree-frog *Phyllomedusa sauvagii*. *Proceedings of the Zoological Society of London* 2010, 893-7.

Budgett, J.S. (1899). Notes on the Batrachians of the Paraguayan Chaco, with observations upon their breeding habits and development, especially with regard to *Phyllomedusa hypochondrialis* Cope. Also a description of a new genus. *Quarterly Journal of the Microscopical Society* (N.S.) 42, 305-33.

Downie, J.R. (1997). Glasgow's neotropical frog connection. *The Glasgow Naturalist* 23(2), 54.

Downie, J.R. (2005). In cold blood: tales of a herpetologist. *The Glasgow Naturalist* 24(3), 56-9.

Downie, J.R. & Nicholls, B. (2004). Comparative breeding ecology of the frogs *Leptodactylus fuscus* and *Physalaemus pustulosus* in Trinidad, West Indies. *Living World* 2004, 12-16.

Frost, D.R. (2011). Amphibian species of the world: an online reference. Version 5.5 (31 January 2011). Electronic database accessible at <http://research.amnh.org/vz/herpetology/amphibia/> American Museum of Natural History, New York, USA.

Hedges, S.B., Duellman, W.E. & Heinicke, M.P. (2008). New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography and conservation. *Zootaxa* 1737, 1-182.

Kenny, J.S. (1969). Amphibia of Trinidad. *Studies on the fauna of Curaçao and other Caribbean Islands* 29, 1-78.

Kokko, H. & Jennions, M. (2010). Ways to raise tadpoles. *Nature* 464, 990-1.

Nokhbatolfighahai, M., Downie, J.R., Clelland, A.K. & Rennison, K. (2005). The surface ciliation of anuran embryos and early larvae: patterns, timing differences and functions. *Journal of Natural History* 39, 887-929.

Nokhbatolfighahai, M., Mitchell, N.J. & Downie, J.R. (2010). Surface ciliation and tail structure in direct-developing frog embryos: a comparison between *Myobatrachus gouldii* and *Pristimantis* (= *Eleutherodactylus*) *urichi*. *Herpetological Journal* 20, 59-68.

Pyburn, W.F. (1980). The function of eggless capsules and leaf in nests of the frog *Phyllomedusa hypochondrialis* (Anura: Hylidae). *Proceedings of the Biological Society of Washington* 93, 153-67.

Warkentin, K.M. (1999). The development of behavioural defenses: a mechanistic analysis of vulnerability in red-eyed tree frog hatchlings. *Behavioural Ecology* 10, 251-62.