

## Egg clutches and predation of *Phyllomedusa azurea* (Anura, Hylidae) nests in a temporary pond in Central Brazil

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Although studies of arboreal clutches in amphibians are increasing (Vaira, 2001; Rodrigues, Uetanabaro and Lopes, 2007), several aspects of such reproductive anuran mode remains poorly known. Some of these aspects are related to clutch and egg survival. Anuran reproductive modes that involve oviposition outside aquatic habitats present new challenges regarding clutch hydration and new forms of predation or parasitism, especially by insects (Heyer, 1969; Magnusson and Hero, 1991; Duellman and Trueb, 1994).

Neotropical frogs belonging to the genus *Phyllomedusa* lay their eggs on leaves, which they fold to form a closed nest (Rodrigues, Uetanabaro and Lopes, 2007), and add gelatinous capsules which are used to seal the leaf, avoid water loss and facilitate complex physiological interactions (Alcaide et al., 2011). Tadpoles then emerge and drop into the water below to complete their development (Zug, Vitt and Caldwell, 2001). The widespread species *Phyllomedusa azurea* Cope, 1862 occurs in open formations of the Chacoan regions of Bolivia, Paraguay and northern Argentina, and in the savanna habitats of Central Brazil (Caramaschi, 2006; Prado et al., 2008; Calderon et al., 2009). It engages in prolonged breeding and is usually found throughout

the rainy season in open habitats associated with ponds (Rodrigues, Uetanabaro and Lopes, 2007; Freitas et al., 2008). Males vocalize on perches in the shrubby vegetation, and amplexant couples commonly lay their eggs on the same shrubs (Zug, Vitt and Caldwell, 2001; Costa, Guimarães and Bastos, 2010).

In the Brazilian Cerrado, Rodrigues, Uetanabaro and Lopes (2007) observed some predated nests of *P. azurea*, but did not identify any predator species. Freitas et al. (2008) observed a predation event by Hemiptera. However, little is known about predation rates and predator species that attack arboreal clutches of this species, and even less has been studied about herbivores that affect nests quality and eggs survival. Here we provide a predation report on clutches of *P. azurea* and the leaves used as oviposition sites in a Cerrado habitat. We also describe the egg laying sites of the species.

We actively searched for clutches of *P. azurea* in a disturbed Cerrado area in Brasília, Distrito Federal, Brazil (15°56'55.19"S, 47°55'55.60"W) between 12 and 17 February 2013. The clutches were located along the shores of a large temporary pond (maximum width: 12 m; maximum length: 60 m; maximum depth: 0.5 m). The pond was located near an off-road, between Cerrado *sensu stricto* and cattle pasture. The vegetation was mainly a composition of native and exotic shrubs (*Ricinus communis*), and exotic grass (*Brachiaria* spp.; *Andropogon* spp.), which colonized the surroundings, reaching a height of no more than three meters.

We marked each shrub with a clutch and every individual clutch. We checked the area and each clutch daily for predation events or new breeding records. We measured the following variables to describe the oviposition site and the egg clutches: 1) distance to margin (from the clutch to the water's edge); 2) presence of other clutches on the same plant; 3) distance to the nearest clutch; 4) clutch height from the ground; 5) number of eggs per clutch; 6) leaf length and width (with clutch); 7) total shrub height; 8) sun exposition at

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**Figure 1.** The most common shrub for oviposition, *Pfaffia glomerata*, with two egg clutches of *Phyllomedusa azurea*.

**Table 1.** Description of oviposition sites and egg clutches from *Phyllomedusa azurea*.

| Variable                  | Mean $\pm$ SD      | Maximum | Minimum | n  |
|---------------------------|--------------------|---------|---------|----|
| <b>Oviposition site</b>   |                    |         |         |    |
| Clutch height (cm)        | 46.00 $\pm$ 32.58  | 160.00  | 5.00    | 31 |
| Shrub height (cm)         | 110.23 $\pm$ 47.75 | 180.00  | 18.00   | 31 |
| Distance to pond (cm)     | 57.64 $\pm$ 65.30  | 310.00  | 0.00    | 31 |
| Distance to clutches (cm) | 57.93 $\pm$ 87.56  | 330.00  | 3.00    | 30 |
| Leaf length (cm)          | 12.63 $\pm$ 11.14  | 71.00   | 5.50    | 31 |
| Leaf width (cm)           | 3.25 $\pm$ 0.85    | 5.00    | 2.40    | 31 |
| <b>Egg clutch</b>         |                    |         |         |    |
| Clutch weight (g)         | 1.08 $\pm$ 0.38    | 1.90    | 0.50    | 19 |
| Clutch length (cm)        | 4.22 $\pm$ 1.59    | 9.50    | 2.53    | 19 |
| Upper gelatinous mass     |                    |         |         |    |
| width (cm)                | 1.07 $\pm$ 0.44    | 1.96    | 0.10    | 20 |
| Lower gelatinous mass     |                    |         |         |    |
| width (cm)                | 0.66 $\pm$ 0.31    | 1.50    | 0.24    | 19 |

midday; 9) shrub isolation (whether or not other shrubs touched it); 10) clutch length (only eggs and gelatinous mass along the leaf); 11) clutch weight (only eggs and gelatinous mass); 12) egg diameter (with and without capsule); 13) upper and lower gelatinous mass width (gelatinous mass sealing the top and the bottom of the leaf). All signs of disturbance to the clutches were recorded. We also report the shrub species in which clutches were found.

We found 31 active egg clutches and all of them were in single living green leaves (Figure 1). Six shrub species were used and the most frequent species was *Pfaffia glomerata*, (Amaranthaceae; n=23). Although we did not measure shrub availability, *P. plomerata* was by far the most abundant in the study area. Other shrub species chosen for oviposition were: *Celosia* sp (Amaranthaceae; n=2), *Hyptis suaveolens* (Lamiaceae; n=2), *Amaranthus lividus* (Amaranthaceae; n=2), *Emilia fosbergii* (Asteraceae; n=1) and *Panicum maximum* (Poaceae; n=1); which were common species in the study area. We suggest that *P. azurea* is an opportunistic species regarding leaf choice for oviposition, since it lays its eggs on different shrub species, in proportion to their availability in the habitat.

Clutches could be found alone in a shrub (n=11) or together with up to five others (n=20). The study was conducted during the end of the wet season and the pond was drying rapidly; therefore the distance of each clutch to the water ranged from 0 (just hanging above water) to 310 cm (n=31). The majority of the clutches were located receiving direct sun-light at mid day (n=25), with few of them in shaded areas (n=6). Clutches could be in isolated shrubs (n=11) or on shrub aggregations (n=20).

We collected data on the number of eggs laid in each clutch (Mean $\pm$ SD) and, in agreement with the values provided by Costa (2008) of 65.41 $\pm$ 16.27 eggs per clutch (n=60), we found a value of 63.5 $\pm$ 6.34 (range=48–74) eggs per clutch (n=20). These values are much smaller than the data reported by Rodrigues, Uetanabaro and Lopes (2007) of 103 $\pm$ 27 (range=50–142) eggs per clutch (n=14). However, these authors counted undeveloped eggs from collected females, explaining the divergence in our results. Based on our data, the clutch size of *P. azurea* is smaller than closely related species, such as *P. hypochondrialis* (range=44–110; Pyburn and Glidewell, 1971), *P. rohdei* (100 $\pm$ 19, n=30; Wogel, Abrunhosa and Pombal, 2005); and larger than *P. megacephala* (range=5–52, n=37; Oliveira, Nogueira and Eterovick, 2012), *P. oreades* (23.14 $\pm$ 6.74, n=7) and *P. ayeaye* (22.43 $\pm$ 5.26, n=7) (Alvares, 2009). Data on clutch length, weight, and upper and lower gelatinous mass width were first described in this study (Table 1).

Oviposition site characteristics varied widely, especially shrub and clutch height (Table 1). Leaf length and width were similar, except for a clutch found on a 71 cm long leaf of a Poacea plant (*Panicum maximum*). Not all egg clutch variables could be measured, mainly due to events of predation or natural failure.

Nine of 31 (29.0%) clutches were found with signs of predation or leaf herbivory. This rate is similar to the predation rate found for the Amazonian treefrog *Phyllomedusa tarsius*, but far less than the rates found for *P. bicolor* (61.0%) and *P. tomopterna* (59.0%) (Neckel-Oliveira and Wachlevski, 2004). We found three predator species using the clutches or nests in some way: ants (Hymenoptera), fly larvae (Diptera) and caterpillars (Lepidoptera).

We found fly larvae and ants (sub-family Formicinae, *Brachymyrmex* sp.) consuming the eggs themselves, whereas the caterpillar were feeding on the leaves. However, we observed that clutches located on damaged leaves by caterpillars are more prone to drying. Most clutches with caterpillars presented partial drying and egg mortality, suggesting that caterpillars cause indirect mortality through compromising nests quality.

We collected and hand-raised in laboratory, under ambient conditions of humidity and temperature, the caterpillars and fly larvae. After metamorphosis, all adults were fixed in alcohol for posterior identification.

The fly larvae were identified as Sarcophagidae, a biologically very diverse family of muscoid flies (Pape, 1996). Larvae of some flesh-eating fly species have been related to adult or tadpole of anurans as myiasis-causing flies in Neotropics, especially those of *Notochaeta* Aldrich, 1916 (Crump and Pounds, 1985; Hagman, Pape and Schulte, 2005; Eizemberg, Sabagh and Mello, 2008; Mello-Patiu and Luna-Dias, 2010; Travers and Townsend, 2010). Predation of turtle and lizard eggs has been related to species of *Tripanurga* and *Eumacronychia*, respectively, but there was no available record of predation on amphibian eggs among the Sarcophagidae until now (Lopes, 1982; Mullen, Trauth and Sellers, 1984; Trauth and Mullen, 1990; Pape, 1996; Bolton, Marshall and Brooks, 2008).

Clutches of *P. azurea* infested by fly larvae were placed in a jar for pupation. Only two adult flesh-eating flies emerged, a male of *Helicobia morionella* (Aldrich, 1930) and a female of *Sarcodexia lambens* Wiedemann, 1830. *H. morionella* has been collected in carrion traps or carcasses as typical saprophagous/necrophagous species, while *S. lambens* has been recognized as a species with great ecological plasticity, reported even as causing myiasis in amphibians and humans (Leão et al., 1996; Hagman, Pape and Schulte, 2005; Fernandes, Pimenta and Fernandes, 2009). Therefore, this rich substrate can be an important alternative resource for maintaining populations of several scavenger species in the absence of any carrion for development of their

larvae, and a resource that may be shared by more than one species.

The caterpillar was identified as being from the Crambidae family, this micro-moth group is known to form leave shelters and eat the leaf from the protection of the structure built (Pullin, 1995). We suppose the caterpillar used an already sealed leaf to save time and energy.

There are few studies regarding predation events in Anura nests and our data on nest predation are clearly too anecdotal for comment, but offer considerable scope for further investigation. This is the first report for such interaction between leaf predation and egg mortality in an arboreal breeding frog.

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