Larval cannibalism in *Phyllobates* poison frogs

Roberto Márquez[1,2]

[1] *Department of Ecology and Evolutionary Biology and Michigan Society of Fellows, University of Michigan. Ann Arbor, MI. 48109. USA*

[2] *Department of Ecology and Evolution, University of Chicago. Chicago, IL. 60637. USA*

Correspondence: Roberto Márquez. Department of Ecology and Evolutionary Biology. University of Michigan. Ann Arbor, MI. 48109. USA. marquezr@umich.edu

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Ethics Statement

Frog rearing protocols were approved by the University of Chicago and the University of Michigan's Institutional Animal Care and Use Committees (UChicago protocol # 72416, UMich protocol # PRO00010325).

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Conflicts of interest

The author reports no conflicts of interest.

Abstract

Cannibalistic behaviour between tadpoles of dendrobatoid poison frogs has been observed in several species with complex parental care dynamics, leading to the idea that it may have played a role in the evolution of parental care. However, the existence or characteristics of this behaviour remain largely unknown beyond a handful of well-studied species. I report direct and indirect observations of larval cannibalism in two species of the genus *Phyllobates*, which seem to occur less frequently than in better studied species, and are possibly associated with body size differences. Beyond expanding the phylogenetic breadth of tadpole cannibalism among poison frogs, my observations point to interesting aspects of this behaviour, such as its apparently plastic and continuous nature, and highlight the importance of studying other lineages to understand its drivers and effects on poison frog evolution.

Keywords: Tadpole cannibalism, Dendrobatidae, Parental care

1. INTRODUCTION

Predation of conspecifics (i.e. cannibalism) is widespread among animals (Fox 1975; Elgar and Crespi 1992). This behaviour is prevalent among amphibian tadpoles, where it has been associated with the resource-limited environments (e.g. ephemeral pools) in which they develop (Polis and Myers 1985; Crump 1990, 1992). Although tadpole cannibalism has been studied in a variety of contexts, such as kin recognition (Pfennig et al. 1993; Pfennig 1997; Gray et al. 2009), foraging behaviour (Caldwell and de Araujo 1998), phenotypic plasticity (Pfennig 1990, 1992), neuroethology (Fischer et al. 2020), and parental care (Summers 1999; Downie et al. 2001; Brown et al. 2009; Rojas 2014), its prevalence across the amphibian phylogeny is not well known.

Larval cannibalism has received considerable attention in Dendrobatoid poison frogs (families Aromobatidae and Dendrobatidae). Tadpoles of multiple species in this group develop in small, resource-poor pools such as puddles in the forest floor, or water-filled plant structures, also known as phytotelmata (e.g bromeliad axels, tree holes, fallen seed husks and petioles). Dendrobatoids lay eggs outside the water, and exhibit considerable parental care, which involves guarding eggs and transporting tadpoles to water upon hatching. In some species parental care extends up to metamorphosis, with females providing infertile eggs to feed their tadpoles (Burst 1993; Weygoldt 1980, 1987; Myers and Daly 1983; Summers and McKeon 2004). Within this group tadpole cannibalism has been reported almost exclusively in a group of closely related genera, namely *Adelphobates* (Caldwell and de Araujo 1998), *Andinobates* (Suárez-Mayorga 1999; Cáceres 2012), *Dendrobates* (Gómez 2006; Gray 2009; Rojas 2014), *Oophaga* (Dugas et al. 2016), and *Ranitomeya* (Summers 1999; Poelman and Dicke 2007; Brown et al. 2011; Schulte et al. 2011; Acosta et al. 2013; Fig. 1). Species in these genera usually display higher levels of parental investment per individual offspring than other dendrobatoids: They lay smaller clutches, often deposit tadpoles individually in phytotelmata, and in many cases extend parental care until metamorphosis. This has lead to the idea that the evolution of increased parental investment may be related to larval cannibalism in the face of limited resources (Summers 1999; Summers and McKeon 2004; Carvajal-Castro et al. 2021).

Despite the sustained interest in larval cannibalism over the past few decades, its prevalence and phylogenetic distribution within dendrobatoid frogs remains largely unstudied. In fact, the existence and characteristics of this behaviour have only been evaluated in one species outside the clade formed by the genera named above (Fig 1): *Mannophryne trinnitatis* (Downie et al. 2001). Although in some cases cannibalism has been considered absent in a handful of species (Carvajal-Castro et al. 2021), this has been based on anecdotal observations of conspecific tadpoles coexisting without apparent aggression. Thus it remains unclear whether cannibalism occurs in these species. Here I report an instance of larval cannibalism in captive-bred individuals of *Phyllobates aurotaenia,* as well as indirect evidence of the same behaviour in *P. terribilis*, both of which occurred incidentally while conducting research on other aspects these species' biology.

2. METHODS AND RESULTS

The frog colonies used in this study were founded using animals obtained from the pet trade in the USA. Adult frogs were kept in groups of 2-6 animals in terrariums and allowed to breed freely. Tanks were checked daily for new egg clutches, which were removed from the tank and reared in petri dishes kept within a sealed container with a moist paper towel to maintain high humidity. After hatching, tadpoles were transferred to 250 or 500ml plastic cups filled with reverse-osmosis (RO) water infused with almond leaf extract, and left to acclimate for three days. On the fourth day post-hatching, one pellet of Frog and Tadpole Bites (Pisces Pros, upc. 788459100303) dusted with Micron Fry Food (Sera, cat. no. 00720) was offered per tadpole. New food was offered when all pellets in a cup had been consumed, and once a week droppings and leftover food were sucked out with a pipette, and new pellets were added. Containers were covered by a lose-fitting lid to avoid excessive evaporation.

Tadpoles were reared in groups of 2-8, which in most cases were made up of full siblings from the same clutch, although in some cases tadpoles from different clutches were reared together. On one occasion, a newly hatched tadpole of *P. aurotaenia* (stage 24-25 *sensu* Gosner, 1960) was moved to a cup with a single stage 34 conspecific that had been last offered food six days prior. Approximately one hour later I observed the older tadpole insistently swimming around and inspecting the hatchling. Soon after, it proceeded to attack the hatchling, aggressively biting its tail. Initially the hatchling was able to turn lose, but the large one persisted until the hatchling became motionless and did not display a detectable heartbeat at plain sight. Over the following ~20 minutes the large tadpole consumed its prey in its entirety. Figure 2A-E show frames taken from a video capturing part of the event. In addition to the event described above, in three occasions I noticed that *P. terribilis* tadpoles being reared in groups with their clutch-mates went missing between daily checkups. Efforts to locate the missing individuals outside of their enclosures were unsuccessful. In view of this, and given the fact that individuals were checked daily and appeared to be in good health, I consider cannibalism to be the most likely explanation for their disappearance, instead of other possibilities such as escape or death due to disease, as dead/escaped tadpoles would have been noticed before decomposing. The missing tadpoles were in all cases between Gosner stages 25-32, and were noticeably smaller than the largest of their clutch mates despite being the same age.

3. DISCUSSION

In this note I report observations of larval cannibalism in two species of poison-dart frog. It is important to recognise that my observations did not occur in the natural context in which these species' behaviours have evolved, since they were made in captivity. That being said, they demonstrate that *P. aurotaenia* and (probably) *P. terribilis* tadpoles are anatomically and behaviourally capable of engaging in cannibalistic behaviour, and that they do so under at least some circumstances that can be relevant in nature, as discussed below. Furthermore they provide the foundation for further work on the ecology and evolution of larval interactions and parental care in poison frogs.

All instances of cannibalism reported here involved predatory tadpoles that were noticeably larger than their prey, suggesting that size disparity may be a key element in the occurrence of cannibalism in *Phyllobates.* This falls in line with previous work on several cannibalistic species (Claessen et al. 2004; Ibáñez and Keyl 2010), including other poison frogs (Rojas 2014; Fouilloux et al. 2022), and highlights the facultative, opportunistic nature of larval cannibalism in poison frogs (Caldwell & De Araújo 1998). *Phyllobates* males transport groups of up to ~10-20 tadpoles, which they are though to deposit in groups at water sources on or close to the forest floor, such as fallen palm bracts and petioles or hollowed logs, that may already be inhabited by conspecifics (Silverstone 1976; Myers et al. 1978; R. Márquez *pers. obs*.). In view of this, I suggest two main situations that may trigger cannibalism in *Phyllobates terribilis* and *aurotaenia* tadpoles (and perhaps those of other species): First, as has been suggested for other species (e.g Rojas 2014), if tadpoles are deposited in a pool already inhabited by older, larger conspecifics, they are at risk of being consumed. Second, when competition for resources between similarly-aged tadpoles inhabiting the same water body (including same-clutch siblings) leads to some individuals growing more slowly than others, and eventually being cannibalised by their larger conspecifics. Clutches reared communally in captivity often display marked body size variation among tadpoles (Fig 2F), suggesting that this is an ecologically relevant scenario. In both cases food scarcity is likely to promote cannibalism, both by directly motivating larger tadpoles to attack their smaller conspecifics, and by promoting stronger intraspecific competition, which in turn leads to greater variance in body size among the tadpoles inhabiting a pool, and increases the likelihood of cannibalism. Further research on the reproductive behaviour of adults and the ecological interactions between tadpoles inhabiting the same pool should help illuminate the relative frequency of both situations, as well as the relationship between cannibalism and the evolution of parental care strategies in *Phyllobates*.

The existence of larval cannibalism in *Mannophryne trinitatis* (Downie et al. 2001), *Phyllobates aurotaenia*, and *P. terribilis* (this study) could, at first glance, appear to challenge to the idea that larval cannibalism is a driver of increased parental investment, since these three species are thought to invest less in parental care than all others where larval cannibalism has been reported, and similarly to species where this behaviour has not been observed (Summers et al. 1999; Summers and McKeon 2004; Carvajal-Castro et al. 2021). However, considering that most examined dendrobatoid tadpoles have omnivorous or predaceous diets (Lehtinen et al. 2004; Ryan and Barry 2011), which in some cases appear indiscriminate (Caldwell and de Araujo 1998), it is possible that tadpoles of most species possess the anatomical and neural machinery required to attack and consume conspecifics. Instead of the discrete presence/absence of cannibalism, species may mostly vary in the conditions that trigger this behaviour (e.g. size differences, food scarcity), and the ease with which it is triggered. The fact that I have observed at most four cannibalistic interactions over eight years of *Phyllobates* captive breeding efforts, while frequent observations of this behaviour have been amply reported in other groups such as *Ranitomeya* (Summers 1999; Poelman and Dicke 2007; Schulte et al. 2011; Acosta et al. 2013; Fischer et al. 2020) or *Dendrobates* (Gómez 2006; Gray et al. 2009; Rojas 2014; Fouilloux et al. 2022), both in the lab and in the field, falls in line with this idea.

Larval cannibalism has been recognised as an important factor in the ecology and evolution of a group of closely-related poison frog genera with more specialised parental care than their relatives. My observations of this behaviour outside this group not only expand the phylogenetic breadth of this behaviour (Fig. 1), but also suggest interesting avenues for future research and reflection around it, such as the degree to which cannibalism can be modelled as a continuous or threshold trait (*sensu* Wright 1934), rather than a binary one, or the environmental and ecological contexts that trigger its expression. Further inquiries of larval cannibalism in other branches of the dendrobatoid phylogeny will certainly help illuminate these and other aspects of the ecology and evolution of larval cannibalism in poison frogs.

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Figures

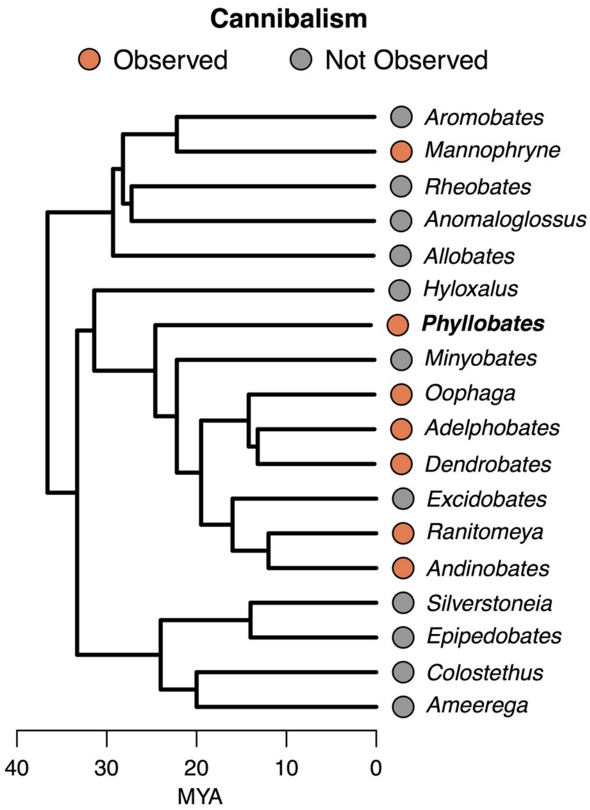

Figure 1. Genera of dendrobatoid frogs where tadpole cannibalism has been observed, with *Phyllobates* highlighted in bold. The topology follows (Grant et al. 2017), and divergence times follow Santos et al. (2014), Guillory et al. (2019), and Douglas, Márquez & Tarvin (*in prep*). Character states were obtained from the literature (see the Introduction for citations), and the figure was plotted using the *plot.phylo* and *tiplabels* functions in the R package ape (Paradis and Schliep 2019).

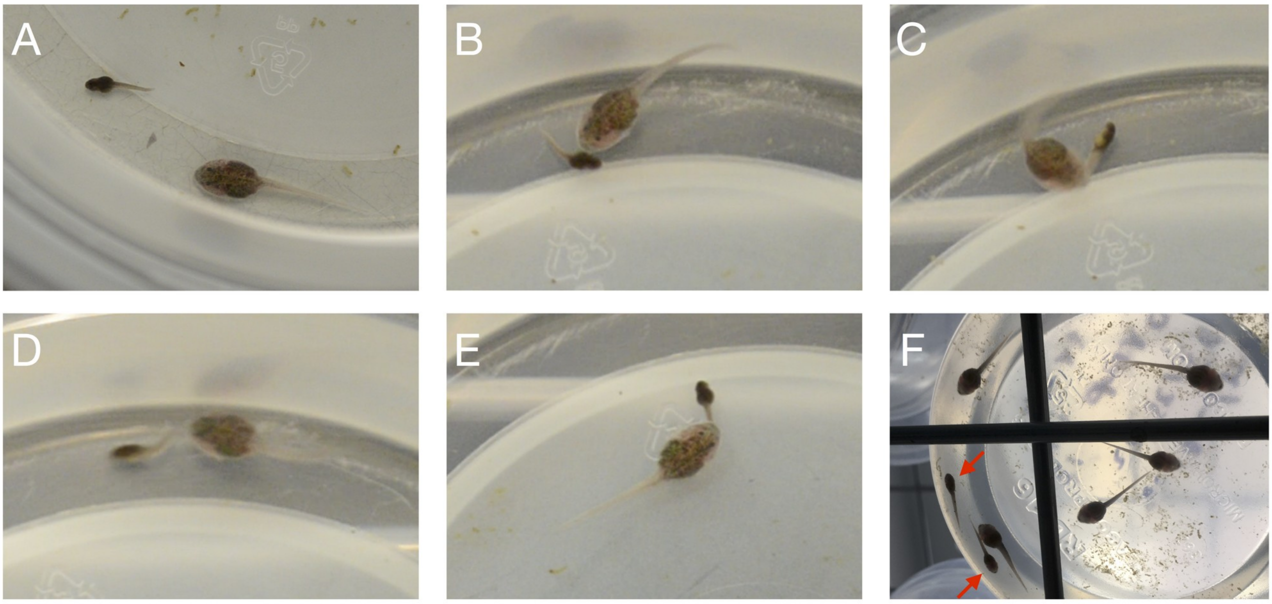

Figure 2. Larval cannibalism in *Phyllobates aurotaenia* (A-E) and within-clutch body size variation in *P. terribilis* (F). After being placed in the same cup, a stage 34 *P. aurotaenia* inspected (A), attacked (B, C), and pursued (D) a recently hatched conspecific (stage 24-25), before eventually killing and consuming it (E). Panel F illustrates body size variation between communally-reared *P. terribilis* tadpoles of the same clutch, presumably due to intraspecific competition. The arrows indicate two individuals noticeably smaller than the rest, despite being the same age.

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