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Diet and tadpole transportation in the poison dart frog *Ameerega trivittata* (Anura, Dendrobatidae)

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Diet and transportation of tadpoles by *Ameerega trivittata* was studied in the eastern Amazon basin. A total of 56 specimens (48 males and 8 females) were sampled, 44 out of which had quantifiable stomach contents. Forty males were recorded to carry between 1 and 18 tadpoles. Forty pools were measured and sampled for tadpoles and odonate naiads, a putative tadpole predator. Myrmicine ants predominated in the diet of males, putatively leading to higher concentrations of alkaloids beneficial during tadpole transport. No relationship was found between male size and the number or size of tadpoles transported, and between pool size and tadpole abundance. The number of tadpoles in the pools was negatively related to the abundance of odonate naiads.

Key words: dendrobatid, flushing, pools, predation, Odonata

While parental care is beneficial for offspring, it usually involves costs for the parents (Trivers, 1972; Clutton-Brock, 1991; Gross, 2005; Kidd et al., 2012). In neotropical dendrobatids (Anura, Dendrobatidae), complex parental care makes reproduction relatively costly (Trivers, 1972; Summers, 1992; Duellman & Trueb, 1994). The survival of tadpoles depends on their successful transfer to pools, as well as pool characteristics such as size (Peltzer et al., 2003) and the presence of predators (Azevedo-Ramos et al., 1992; Hero et al., 1998; Rodrigues et al., 2010). Diet plays a further role in providing parental care. Dendrobatids are considered ant-feeding specialists (Toft, 1980), and their diet appears to provide the alkaloids used for defence against predators (Daly, 2004). The diet in dendrobatids also differs between sexes (Forti et al., 2011). For example, *Oophaga pumilio* females feed more on ants than males, which might be linked to higher alkaloid concentrations beneficial for parental care, considering that transporting tadpoles to pools may expose them to higher predation risk (Donnelly, 1991; Saporito et al., 2010). This is also suggested for *Dendrobates tinctorius*, where males are more yellow than females, possibly a favourable trait because males are exposed to predators for longer periods during tadpole transport (Rojas & Endler, 2013).

Here, we studied the composition of the diet and tadpole transportation of *Ameerega trivittata* in the eastern Amazon basin. We addressed three questions: (i) Do males and females differ in diet composition? We expect that males, the sex which performs tadpole transportation in this species, consume larger quantities of ants in comparison to females. (ii) Is the number and volume of tadpoles transported by a male related to male body size? (iii) Is the number of tadpoles in a pool influenced by the surface area and the presence of odonate naiads? We predict that that tadpoles will be more abundant in larger water bodies an in pool with less odonate naiads.

Ameerega trivittata (the three-striped poison frog) is a diurnal dendrobatid species (Grant et al., 2006) inhabiting forest floors in Venezuela, Guyana, Suriname, and the Amazonian basin of Brazil, Bolivia, Colombia and Peru (Frost, 2014). They are characterised by a black dorsum with bright yellow to green marbling (Roithmair, 1994) and a toxic skin (Daly et al., 1987). The study was conducted in Juruti municipality (02^{o}09'09" S, 56^{o}05'42" W), eastern Amazonian basin, Brazil. The climate of the region is tropical moist (Peel et al., 2007). Temperatures range between 22.5°C and 31°C, with annual precipitation of about 2200 mm. The rainy season lasts from February to April, while the driest season is from August to October (Moraes et al., 2005).

Sampling was carried out from January to March 2011, covering 58 days of daily fieldwork with an effort of 6 to 8 hours per day. All specimens were captured, measured (snout-vent length, SVL) and weighed. Only adults (≥35 mm, Roithmair, 1994) were considered. Stomach contents were extracted using the flushing method (Legler & Sullivan, 1979; Leclerc & Courtois, 1993; Born et al., 2010), preserved in 70% alcohol and identified to the Order level. Tadpole-carrying males were captured when they were near to a pool. The number of tadpoles was

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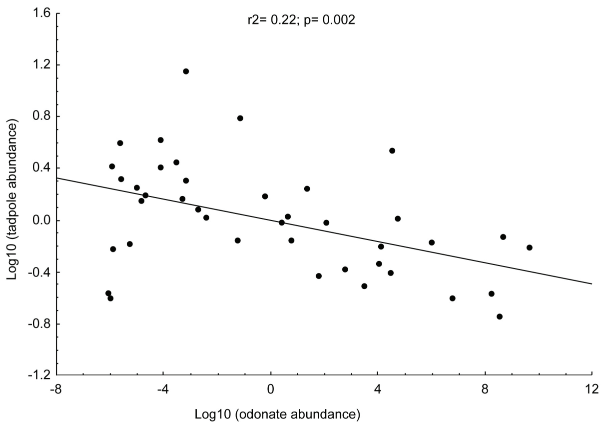

Fig. 1. Partial regression illustrating the isolated effect of the abundance of odonate naiads on the abundance of *Ameerega trivittata* tadpoles in pools in Juruti, Pará, Brazil.

counted, and their size measured (body length × width × height). After measurements, males were returned to the site of capture and tadpoles were released in the nearest pool. The size of 40 representative pools was estimated by calculating the area of the ellipse as *A*=π*ab*, where *a*=the major semi-axis of the ellipse (=length) and *b*=minor semi-axis of the ellipse (=width). The number tadpoles and odonate naiad nymphs were in these pools were recorded by sweeping the whole pool with a 3-mm sieve mesh, including the substrate and the leaf litter of the bottom (Gascon, 1991; Shaffer et al., 1994). Each pool was sampled for at least 30 minutes, and sampling was terminated when no specimens (tadpoles or odonate naiads) were found in a 5–10min interval (Rodrigues et al., 2010).

The influence of sex on diet composition was tested using the simplified Morisita-Horn index of overlap (Horn, 1966):

$$CH=(2 \sum P_{ij} \times P_{ik}) \div [(\sum P_{ij}2/N_j2) + (\sum P_{ik}2/N_k2)]\ N_j \times N_k$$

where *CH*=Simplified Morisita-Horn index, *Pij*=the proportion of item *i* in the total number of items ingested by the females, P_{ik}=the proportion of item *i* in the total number of items ingested by the males, *n*=total number of items used. This index is used to quantify overlap in diets, and varies from 0 to 1, with higher values indicating a higher degree of overlap. This index was chosen because it is applicable to varying sample sizes, and because of its wide use (Wolda, 1981; Marshal et al., 2012; Preti et al., 2012). Stomach contents were analysed using the dominance index (Benneman et al. 2006). Here, dominance is related to the number of times that a given item is most common, expressed as a percentage of the total number of stomachs analysed. The frequency of occurrence (*FoR*%) was also calculated for each food item, expressed as the percentage of the samples in which the item was observed. A linear regression was used to determine whether abundance and volume of tadpoles is related to male body size (SVL) and a body condition index (BCI, Anderson & Neumann, 1996). A multiple linear regression was used to test whether abundance of tadpoles in pools is related to pool size and abundance of odonate naiads. Pool area and tadpole abundance values were transformed ($Log_{10}(N+1)$) to conform with

Table 1. Diet of males (*n*=48) and females (*n*=8) *Ameerega trivittata* on the municipality of Juruti, Pará, estimated by the flushing method. *FoA* - absolute frequency of occurrence; *FoR*% - relative frequency of occurrence; % D - Dominance. The most dominant orders are highlighted in *italic*.

Prey Category	Males			Females		
	FoA	*FoR* (%)	D (%)	*FoA*	*FoR* (%)	D (%)
Acari	4	10.81	2.70	-	-	-
Araneae	5	13.51	-	1	14.29	-
Chilopoda	1	2.70	-	2	28.57	-
Coleoptera	24	64.86	-	6	85.71	14.29
Diptera	4	10.81	-	2	28.57	-
Hemiptera	1	2.70	-	-	-	-
Hymenoptera	35	94.59	*64.86*	6	85.71	14.29
Isopoda	3	8.11	-	-	-	-
Isoptera	19	51.35	27.03	6	85.71	*71.43*
Lepidoptera	1	2.70	-	-	-	-
Opiliones	1	2.70	-	-	-	-
Stones	3	8.11	-	-	-	-
Thysanoptera	1	2.70	-	-	-	-
Leaves	20	54.05	2.70	4	57.14	-

a normal distribution. All analyses were conducted using the R v.2.13 software (R Development Core Team 2011)

In total, 48 males (SVL mean=38.0±0.3 mm) and 8 females (SVL mean=44.3±1.4 mm) were captured. Fourteen different items were identified in the stomach contents of 44 specimens (Table 1), and all of them were found in the stomachs of both sexes. There was an overlap of 72% in the diets between sexes (Morisita-Horn index: *CH*=0.72). The most abundant order in the male diet was Hymenoptera (64.86%), whereas Isoptera (71.43%) was predominant in the female diet. Forty males were found carrying between 1 and 18 tadpoles (males SVL: 37.24±1.95 mm, mass: 4.41±0.78 g, *n*=10.8±3.2 tadpoles with mean sizes of 6.0±2.0mm). Neither abundance (p=0.45, SE=28.60, β=0.12) nor size of the tadpoles (p=0.36, SE=0.19, β=0.07) were related to the size of the male transporters or with BCI (r^2=0.03, y=0.12x+1.04). The 40 pools had a mean area of 2.12±2.57 m² and contained between 0 and 16 odonata nymphs (mean=6.85±4.9) and 8 to 533 *A. trivittata* tadpoles (mean=76.65±96.7). The variation in the abundance of tadpoles in the pools was explained by the model (r^2=0.25, $F_{2,37}$=5.6, p=0.005). Partial regressions revealed a negative relationship between tadpole and abundance of naiads (r^2=-0.22, $F_{(1,38)}$=10.58, p=0.002; Fig. 1), without any effect of pool area (r^2=0.001, $F_{(1,38)}$=0.05, p=0.83).

The diet of *A. trivittata* was mainly composed of social insects. Behavioural differences between sexes may be associated with the partitioning of feeding resources within the population. A predominance of hymenopteran prey in male diet and isopterans in female diet was previously recorded in *A. braccata* (Forti et al., 2011), a species for which males are also responsible for tadpole transport (Uetanabaro et al., 2008). Donnelly (1991) also found for *O. pumilio* that the tadpole-carrying sex (females) consumed higher quantities of ants, putatively resulting in higher levels of alkaloids (Saporito et al., 2010). While the composition of male and female diets in *A. trivittata* overlapped, only males had ants as a predominate diet. This could be due to males ingesting prey items rich in alkaloids, contributing to their anti-predator defences during tadpole transportation.

No relationship was found between size and body condition of males and the size and number of tadpoles they carried. In *O. pumilio*, females carry only 1–2 tadpoles at a time during a number of trips to deposit their offspring into a pool (Weygoldt, 1980; Ringler et al., 2013). A similar behaviour was suggested by Acioli & Neckel-Oliveira (2014) for *A. trivittata*, which may account for the lack of a relationship between the size of males and tadpoles being transported.

The inverse relationship between the abundance of tadpoles and odonate naiads was similar to previous findings by Von May et al. (2009), whereas another related species, *Ranitomeya biolat*, tended to deposit tadpoles in pools with fewer naiads. In an experimental study with two hylid species, Van Buskirk (1988) found that the abundance of tadpoles decreased as the number of odonate naiads increased. Male *A. trivittata* might have deposited tadpoles in pools with less naiads, or tadpoles might have been predated by them. Cannibalistic behaviour as observed for e.g., *Dendrobates ventrimaculatus* (Summers & Symula, 2001) could further have contributed to observed tadpole abundance. The abundance of *A. trivittata* tadpoles was not related to pool area. While larger pools would be expected to support larger numbers of individuals (Rosenzweig, 1995), they likely also have a longer hydroperiod, which may favour the presence of predators (Carvalho & Nessimian, 1998; Neckel-Oliveira, 2007). However, other pool variables such as depth and bottom litter may also influence the abundance of tadpoles (Sanderson et al., 2006) and were not measured in this study.

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