

Influence of tail injury on the development of Neotropical elegant treefrog tadpoles

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Abstract. Anuran larvae in aquatic environments are important prey items for several vertebrate and invertebrate species. Besides avoiding predation, there are some strategies that may reduce the physical damage in those tadpoles that survive the predation attempt. For example, the injured tadpole tail can regrow after a predator bite, but few studies have examined the consequences of such injury. We examined the consequences of three levels of injury to the tail and how this influenced development and feeding behavior of tadpoles of the Neotropical elegant treefrog, *Dendropsophus elegans*. We collected spawns and kept them in the laboratory until tadpoles reached Gosner's stages 28 to 35. Then, they were separated in four experimental groups: individuals with tail trimmed in 30, 50 or 70 % of its length, and a control group, with no tail removing. We counted the days until metamorphosis, calculated the Scaled Mass Index (SMI) through weight and length of newly-metamorphosed, and evaluated the feeding frequency to evaluate the influence of tail amputation on them. We found that the time until metamorphosis was positively related to the extent of the amputation, but SMI and feeding behavior were not influenced. As the time to metamorphose is related to the survivorship chances of individuals: i.e., if the aquatic environment is with high density of predators, it would be advantageous to rapidly metamorphose out of the water. However, tail injury delays the metamorphose process, which could influence the survival of the individual.

Keywords. Anuran larvae, *Dendropsophus elegans*, Atlantic rainforest, tail loss, development, feeding.

INTRODUCTION

Most anurans present aquatic larval stages and terrestrial post-metamorphic (adult) life stages, and are susceptible to predators of both environments. In this context, several defensive strategies were already reported for tadpoles in face of predators' attack. For example, tadpoles of *Pelophylax lessonae* can alter their behavior in the pres-

ence of dragonfly larvae (van Buskirk and Arioli, 2002), and tadpoles of *Dryophites crysoscelis* can change the morphology of their tails in order to increase swimming speed, which consequently promotes a higher probability of escaping in a possible attack of predators (McCollum and Leimberger, 1997). Also, Other species rely on visual aspects to avoid predation, such as tadpoles of *Scinax machadoi*, which select background colors to improve

their camouflage (Eterovick et al., 2018; Gontijo et al., 2018), *Pseudacris regilla*, which alter their tail color to avoid predator attacks (Benard, 2006), and *Boana semi-lineata*, which uses aposematic coloration to avoid predation (D’Heursel and Haddad, 1999). Hence, other species, such as *Bufo bufo*, rely on chemical defenses to avoid attacks of predators (Üveges et al., 2019).

Anurans are well known to be centralized in trophic webs (Blanco-Torres et al., 2020) since they are both prey and predators (Rebouças et al., 2013; Rebouças and Solé, 2015). In this way, they evolved several strategies to avoid predation (e.g., Lourenço-de-Moraes et al., 2016; Toledo et al., 2007). In larvae, one of the possible sublethal consequences of a predation attempt is the partial tail loss or injury (Morin, 1985; Touchon and Wojdak, 2014; Wilbur and Semlitsch, 1990), but the consequences of it to individual survival are very variable. For some species, past evidence suggest that it incurs little cost for tadpoles, since they, after escaping the predation, can regenerate the tail completely (Wilbur and Semlitsch, 1990). For example, van Buskirk et al. (2003) observed that tails may play a role as a lure, in which larger tail fins reduced predations in 16 % of the observations. Indeed, although firstly reported that enlarged tail fins enables predator escaping by enabling faster swimming (Smith and van Buskirk, 1995), posterior studies showed that tadpoles with injured tails did not lost speed in relation to those with an intact tails (van Buskirk and McCollum, 2000a). The effect on speed was significant only if large portions of the tails were removed (Hoff and Wassersug, 2000; van Buskirk and McCollum, 2000b). However, for some species tail injuries result in less swimming performance, and consequently a higher predation risk. In *Dryophytes chrysoscelis*, for example, tadpoles with no tail injury presented a survival almost twice as high as those with 75 % of tail loss (Semlitsch, 1990). Also, in *Bombina orientalis* tadpoles presented less survivorship and longer larval period (Parichy and Kaplan, 1992).

Beyond the ecological consequences, tail loss in tadpoles can also present feeding activity modification. Theoretically, if individuals need no regenerate tails after a predation attempt, they should acquire more energy through feeding to reach the maximum of tail length as less time as possible, and consequently reach the full swim performance, which is related to tail shape (van Buskirk and McCollum, 2000b). However, although modification of feeding behavior is already observed in presence of predators (e.g., Feminella and Hawkins, 1994; Pueta et al., 2016), the effects of tail loss on it, which is the most common consequence of predation attempt, still were not observed. Hence, while regenerating the tail, tadpoles are in continuous growth, which *per se* requires

a constant food intake until reach the metamorphosis stage. Thus, the tail injury, and an extra acquisition of nutrients during its regeneration, must affect the feeding-growth-time until metamorphosis balance. It is relevant because tail injuries may impact on the population survivorship coupled with the fact that this species occurs in Atlantic rainforest, one of the most diverse and vulnerable environments of the world, where pandemic diseases (Carvalho et al., 2017) climatic changes (Moura-Campos et al., 2021; Rebouças et al., 2021), habitat fragmentation (Becker et al., 2010; Dixo et al., 2009), and introduced predators (da Silva et al., 2009; de Oliveira et al., 2016; Forti et al., 2017) are threatening endemic anurans.

Therefore, this study evaluates the consequences of tadpole’s tail injuries in a Neotropical anuran species, *Dendropsophus elegans* (Anura; Hylidae), testing the following hypotheses: i) different levels of tail injury result in less healthy newly-metamorphosed; ii) different levels of tail injury increase the time to complete metamorphosis; and iii) tail injury reduces foraging activity of tadpoles.

MATERIALS AND METHODS

Tadpoles of *Dendropsophus elegans* (Fig. 1) were obtained through the maintenance of egg masses collected at Reserva Betary, Iporanga, São Paulo, Brazil. After hatching, each tadpole was kept in an individual aquarium (40 x 45 x 30 cm), to avoid pseudo-replicates and the influence of one individual in another, maintained at room temperature (25 °C), and half of the water was replaced twice a week after tadpoles reach the stage 28. We used tadpoles between Gosner’s (1960) stages 28 and 36 for the experiments. These stages were chosen because they comprehend most of growth and development of anuran larvae (Pfab et al., 2020). Environmental conditions of laboratory were constantly monitored and individuals were observed until metamorphosis. Thus, our experiment began before hatching and finished after metamorphosis. After the experiment, all individuals were released in the original sampling locality.

Tadpole development. To evaluate the influence of tail loss in the size and growth of individuals, we selected tadpoles that measured 25 mm of total length. Individuals were measured with a digital caliper (to the nearest 0.01 mm) and weighted with a digital scale (to the nearest 0.01 g). We then arranged these tadpoles into four groups, following Semlitsch (1990) and Figiel Jr and Semlitsch (1991), representing each of the treatments: i) tadpoles with 30 % of the tail clipped; ii) tadpoles with 50 % of tail clipped; iii) tadpoles with 70 % of tail clipped;

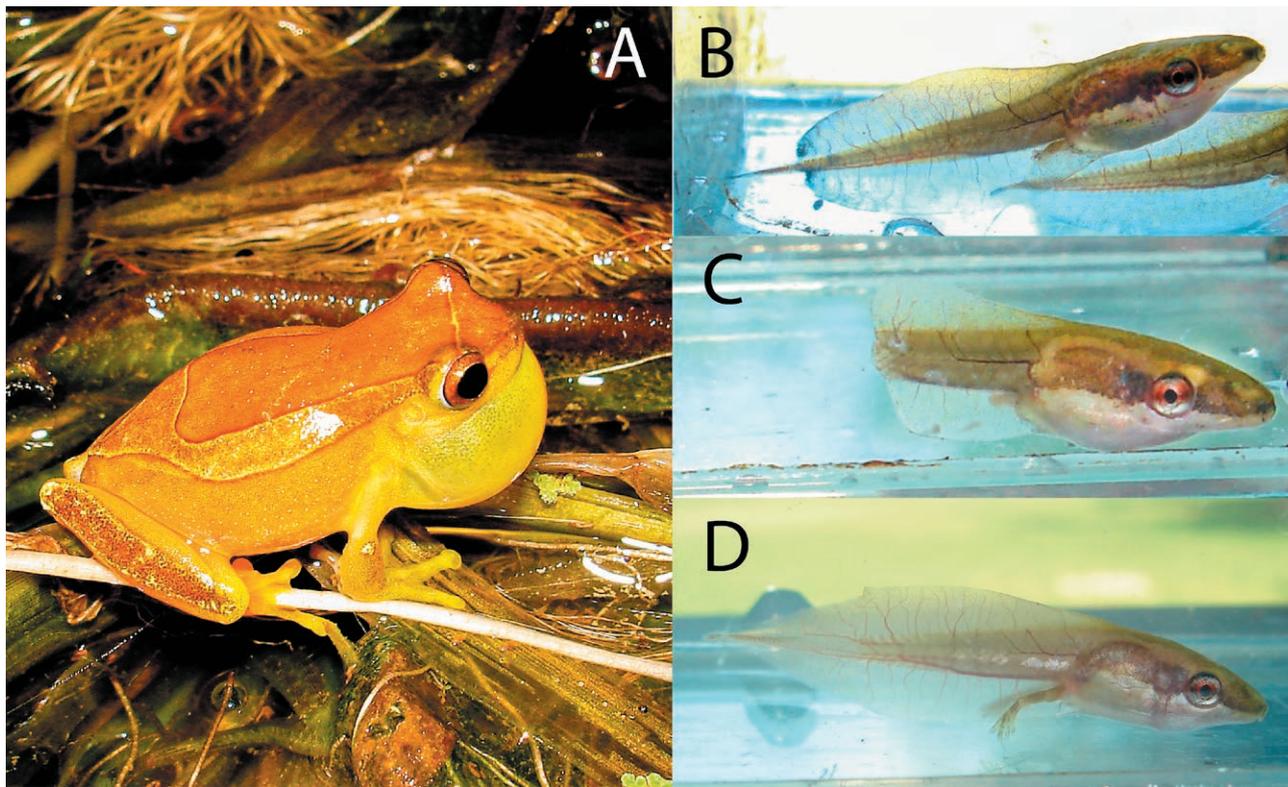


Fig. 1. An adult individual of *Dendropsophus elegans* (A), tadpoles of control (B) and 50 % of the tail clipped treatments (C), and with regenerated tail (D).

and iv) tadpoles with intact tails, which was the control group (Fig 1). Each group contained between 8 and 10 individuals (Table 1), which were isolated in each aquarium. Tail modifications were performed using a sterilized scalpel blade. Individuals in all treatments were equally fed with a standard fish food (extruded AquaLine), with 0.1 g every day. Individuals were observed until the metamorphosis was completed (complete tail absorption), and snout-vent length (SVL) of newly-metamorphosed individuals was measured with the digital caliper and body mass was weighted with the digital scale. Body mass and weight were used to calculate the Scale Mass Index (SMI), which is an index that can be used as a proxy of animals' health and fitness (Peig and Green, 2009).

Foraging. In order to evaluate the influence of partial tail loss in tadpoles foraging, we performed a second experiment also using 10 individuals measuring 25 mm in total length and between Gosner's (1960) stages 28 and 36. These individuals were separated in two treatments: i) individuals with 70 % of tail amputated; and ii) individuals with intact tails, treated as the control. Tadpoles were kept individually in glass jars measuring 6.5 cm in diameter and 6 cm height, with 120 ml of water and 0.1 g

of fish food. After two min of acclimation, tadpoles were observed for 12 min. During this time, the feeding frequency was observed in intervals of 20 s, and during each observation we evaluated if they were feeding or not.

Statistical analyses. Firstly, we used an Analysis of Variance (ANOVA) and a Student's *t* test to evaluate of SMI present difference between treatments (tail amputations of 30 %, 50 % and 70 %; and tail amputation *per se*, respectively). To evaluate the influence of tail injury on SMI and on time until metamorphosis, we ran two Generalized Linear Models analyses (GLM), both using treatment (30 %, 50 % and 70 % of tail amputations and control, coded as 1, 2, 3 and 0, respectively) as predictive variable, the first one with SMI of newly metamorphosed individual as response, and the second with days until metamorphosis as response. Both analyses were performed using gaussian family and identity link. Additionally, we ran other two GLM's, with the same parameters, to evaluate if SMI or days until metamorphosis were influenced by amputation *per se* (all treatments were classified as "amputated", for treatments which the tail was clipped, coded as 1, and "intact" for the control treatment, coded as 0). Finally, in order to evaluate the

influence of tail loss in foraging we also used a GLM, but with quasipoisson family and logit link, considering “treatment” as predictive variable (control, coded as 0, or amputation, coded as 1), and the feeding frequency as response variable.

All models were checked through residuals deviance, and models with more than one predictive variable and collinearity was checked through Variance Inflation Factor (VIF) through the “vif” function of “car” package (Fox & Weisberg, 2019). We considered levels higher than 4 as an indicator of multicollinearity (Hair et al., 2010). Hence, as pos hoc tests, we used estimated marginal means to compare groups of tail-trimmed individuals with the control group through the “emmeans” package (Lenth, 2020). All analyses were carried out in R 4.1.0 (R Core Team, 2021) considering a significance level of 5 %.

RESULTS

During the experiment about tadpole development, we recorded the death of four individuals: one from the control group, one from the 50 % amputation group, and two from the 70 % amputation group. All individuals from the treatment groups presented the tail totally regenerated within 12 days after the beginning of the experiment (Table 1). We observed tail regeneration in all individuals that had their tail clipped (Fig. 2).

The average time until metamorphosis (from eggs until newly-metamorphosed) was 87.5 days for the control group (room mean temperature of 26.5 °C; Table 1). We observed no difference between treatment groups ($F = 0.91$, $P = 0.44$) or between individuals with tail amputation or not ($t = -0.06$, $P = 0.95$). Newly metamorphosed individuals presented an average SMI of 0.148 ± 0.012 , with control group presenting 0.148 ± 0.011 , 30 % group presenting 0.143 ± 0.013 , 50 % group presenting 0.153 ± 0.012 , and 70 % group presenting 0.149 ± 0.014 . During foraging experiment, individuals with injured tail were

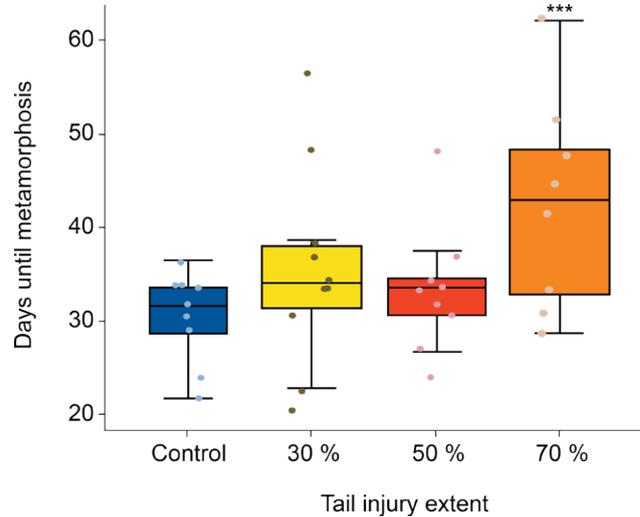


Fig. 2. Days until metamorphosis of *Dendropsophus elegans* tadpoles subjected to four treatments: control (intact tail), and 30, 50 and 70% of tail removal. The top and bottom of the boxes represent the first and last quartiles, the horizontal line within the box represents the median, the whiskers represent the tenth and 90th percentiles. Asterisks represent the category of tail amputation that showed significant reduction of time until metamorphosis.

observed feeding in an average of 36.6 ± 25.9 times, while individuals with no tail injuring were observed feeding in an average of 55.1 ± 22.9 times.

In our analysis, none of the variables presented VIF higher than 4 (SVL = 3.02, weight = 3.35, days until metamorphosis = 1.2). We observed no influence of treatment on SMI or in weight, but treatment presented a significant influence on time until metamorphosis. Amputation *per se* showed no influence in any of our variables. Regarding to foraging, we observed no influence of tail injury on feeding frequency. All model outputs are in Table 2 and estimated marginal means in Table 3.

Table 1. Time until metamorphosis, snout-vent length (SVL) and body mass of newly metamorphosed individuals during experimentation. Values presented as mean \pm standard deviation (minimum – maximum; number of individuals tested; standard error).

Treatment	Time until metamorphosis (days)	SVL (mm)	Weight (g)
Control	29.8 ± 11.5	12.06 ± 0.44	0.15 ± 0.02
	(18.3 – 41.3; 9; 3.83)	(11.62 – 12.49; 9; 0.14)	(0.13 – 0.17; 9; 0.007)
30 %	35 ± 10.8	11.86 ± 0.46	0.14 ± 0.02
	(24.2 – 45.8; 10; 2.98)	(11.40 – 12.32; 10; 0.14)	(0.12 – 0.16; 10; 0.006)
50 %	32.8 ± 7.1	12.13 ± 0.24	0.16 ± 0.02
	(25.7 – 39.9; 9; 2.36)	(11.90 – 12.37; 9; 0.08)	(0.14 – 0.18; 9; 0.007)
70 %	42.0 ± 15.1	12.01 ± 0.44	0.15 ± 0.02
	(26.9 – 57.1; 8; 5.34)	(11.57 – 12.46; 8; 0.14)	(0.13 – 0.17; 10; 0.006)

Table 2. Coefficients of Generalized Linear Model analysis, which considers the percentage of tail injury as a predictor of (1) Scaled Mass Index (SMI) and (2) days until metamorphosis; tail injury *per se* as a predictor of (3) SMI and (4) days until metamorphosis; and (5) tail injury as a predictor of feeding frequency. All models present degrees of freedom = 35 and significant values are in bold.

	Estimate	Std Error	t value	P
(1) SMI ~ % tail injury				
Intercept	0.15	0.004	34.42	<0.001
30%	-0.005	0.006	0.79	0.44
50%	0.005	0.006	-0.81	0.42
70%	0.001	0.006	0.25	0.81
(2) Days until metamorphosis ~ % tail injury				
Intercept	29.78	2.99	9.95	<0.001
30%	5.22	4.13	1.27	0.21
50%	3	4.23	0.71	0.48
70%	12.22	4.36	2.8	0.008
(3) SMI ~ tail injury				
Intercept	0.15	0.004	34.05	<0.001
tail loss	0.0003	0.005	0.05	0.96
(4) Days until metamorphosis ~ tail injury				
Intercept	29.79	3.15	9.56	<0.001
tail loss	6.56	3.6	1.82	0.08
(5) Feeding frequency ~ tail injury				
Intercept	4.01	0.18	22.56	<0.001
tail loss	-0.41	0.28	-1.45	0.17

Table 3. Summary contrasts of Estimated Marginal Means, used as a pos hoc test to compare groups of different levels of tail injury with the control group. Significant value is in bold.

	Estimate	Std. Error	P
SMI ~ % tail injury			
30% - control	-0.005	0.006	0.73
50% - control	0.005	0.006	0.75
70% - control	0.001	0.006	0.98
Days until metamorphosis ~ % tail injury			
30% - control	5.2	4.13	0.44
50% - control	3	4.23	0.79
70% - control	12.22	4.36	0.01
SMI ~ tail injury			
injured - control	0.0003	0.005	0.96
Days until metamorphosis ~ tail injury			
injured - control	6.56	3.6	0.07
Feeding frequency ~ tail injury			
injured - control	-0.41	0.28	0.15

DISCUSSION

We showed that although tadpoles reach metamorphosis with the same weight and size in all classes, the time spent until the end of the metamorphosis tends to increase, and it was significantly longer when 70 % of tail is removed. It means that individuals with a severe damage in tail tend to spend more time under larval stage, which can submit individuals that were already threatened by a predator under aquatic predation pressure for a longer time. Also, it delays the development of adult life stage, and consequently reproduction can be retarded. Therefore, a high predation pressure can influence other life stages of individuals, and in a larger scale, can impair the permanence of a population.

We also observed that the feeding frequency was not significantly higher in the group with tail trimmed. Some similar results were observed in other experiments involving artificial tail removing in tadpoles of *Aquarana catesbeiana*, where individuals also had a delay in growth and development (Wilbur and Semlitsch, 1990). A possible explanation for these observed results is that a predation attempt does not result in increasing of uptake but in reallocation of energy, since feeding presented no increasing, and it consequently could cause a delay in development. Additional studies are necessary to further elucidate the physiology of this possible energy reallocation and verify this hypothesis.

We did not observe influence of tail removal on the SMI of newly-metamorphosed individuals, similarly to what was reported for size in *Osteopilus septentrionalis* (Koch and Wilcoxon, 2019) and *Hoplobatrachus rugulosus* (Ding et al., 2014). However, opposing results were found for other species. For example, in *Bombina orientalis*, for which the time until metamorphosis was the same independently of the tail injury extent, newly metamorphosed individuals were smaller than those without tail injury (Parichy and Kaplan, 1992). Likewise, tadpoles with 55 % of the tail removed resulted in smaller newly-metamorphosed individuals in *Pelobates cultripes* (Zamora-Camacho et al., 2019). Besides, such effect lead to a reduction in the jumping performance of post-metamorphic individuals (Zamora-Camacho and Aragón, 2019), which could expose them to higher risk of predation on land. So, these cases highlight a trade-off: tadpoles will either stay longer in the water, exposed for a longer time to aquatic predators but with newly metamorphosed with an 'ideal' size, with less exposure to terrestrial predators (Semlitsch, 1990; Wilbur and Semlitsch, 1990), or they could leave the water smaller and with some mobility handicaps, which could limit the exposure to aquatic predators but exposing them more to terrestrial predation

in the developmental stage that they are most susceptible to predation (Toledo et al. 2007). In *D. elegans* we observed that the strategy adopted is the first one. Tadpoles threatened by a predator spend more time under larval stage, i.e., reduce the growth rhythm, but reach the same size after metamorphosis, and consequently the same SMI, than unharmed individuals.

We also did not observe change in feeding frequency as a result of tail injury. It probably implies that the tail regeneration was not provided by an extra acquisition of energy – expected by a more frequent feeding. Although these stages (stages 28 until 36) are those when generally tadpoles present the most significant growth and energy uptake (Pfab et al., 2020), we did not observe any difference when the tail was lost. Considering that for some species locomotion is more important than feeding, such as in *Pleurodema thaul* (Pueta et al., 2016) and *Pelophylax lessona* (Steiner, 2007), and that tail fins enable fast swimming (Smith and van Buskirk, 1995), perhaps for *D. elegans* the regeneration of tail is energetically more important than time until metamorphosis. Consequently, there is not an increase in feeding to regenerate the tail, but a reallocation of the energy that otherwise would be used to growth. Thus, such observation supports the hypothesis of a probable reallocation of the energy from the regular development/metamorphosis process directed to tail regeneration. However, different results were reported for other species. For example, in *Ascaphus truei*, a simple clue of predators' presence was enough to modify the foraging in tadpoles, which reduced up to six-folds its foraging activity (Feminella and Hawkins, 1994). Also, similar results were observed for *Rana sylvatica* (Fraker, 2010) and *Rana clamitans* (Fraker, 2008, 2009). It efforts that more studies are necessary to elucidate this process of energy reallocation during larval stage until metamorphosis.

Our experiments showed consequences of predatory events in *D. elegans* tadpoles. Tail injury caused by predators can result in several consequences for the individuals, decreasing their survivorship, affecting tadpole morphology (Nunes et al., 2010), and swimming speed (Figiel Jr and Semlitsch, 1991). Besides, as tadpoles of *D. elegans* remained more time in the larval stage when the tail was injured, this fact may have several consequences, since evolutionary approach until conservation of native populations.

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