INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA – INPA

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

COMO A VULNERABILIDADE À PREDAÇÃO VARIA AO LONGO DA ONTOGENIA DE GIRINOS?

MILLA RAYSSA MARTINS DUTRA NUNES

Manaus - Amazonas

Fevereiro, 2023

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Dissertação apresentada ao Instituto Nacional de Pesquisas da Amazônia (INPA) como parte dos requisitos para obtenção do título de Mestre em Biologia (Ecologia).

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ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 31 dias do mês de Janeiro do ano de 2023, às 09h00min, na Sala nº 13 do PPG-Ecologia (Prédio 140), reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: a Dra. **Daiani Kochhann**, da Universidade Estadual Vale do Acaraú – UVA, o Dr. **Lucas Ferrante de Faria**, egresso do PPG-Ecologia do Instituto Nacional de Pesquisas da Amazônia – INPA e o Dr. **Pedro Paulo Goulart Taucce**, do Instituto Nacional de Pesquisas da Amazônia – INPA, tendo como suplentes o Dr. Marcelo Gordo, da Universidade Federal do Amazonas – UFAM e a Dra. Marina Anciães, do Instituto Nacional de Pesquisas da Amazônia – INPA, sob a presidência da orientadora, a fim de proceder a arguição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** de **MILLA RAYSSA MARTINS DUTRA NUNES**, intitulado: **"COMO A VULNERABILIDADE À PREDAÇÃO VARIA AO LONGO DO DESENVOLVIMENTO ONTOGENÉTICO DE GIRINOS?"**, orientada pela Dra. Cintia Cornelius Frische e co-orientada pelo Dr. Igor Luis Kaefer, ambos do Instituto Nacional de Pesquisas da Amazônia – INPA e UFAM.

Após a exposição, o discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

☒ APROVADO (A) ☐ REPROVADO (A)
☒ POR UNANIMIDADE ☐ POR MAIORIA

Nada mais havendo, a presente ata foi lida, lavrada e assinada pelos membros da Comissão Examinadora.

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SINOPSE

Investigamos como a vulnerabilidade à predação varia ao longo da ontogenia de girinos do anuro *Allobates sumtuosus* por meio de um experimento laboratorial de encontro predador-presa, sem escolha, utilizando como predador a larva da barata d'água *Belostoma stollii*. A partir disso, avaliamos a relação de variáveis de desenvolvimento e crescimento, bem como seus efeitos na relação predador-presa em caso de encontro. Observamos que a vulnerabilidade à predação reduz ao longo do desenvolvimento e crescimento dos girinos (relação linear), contudo girinos em fases tardias podem apresentar maior vulnerabilidade (relação quadrática) considerando suas habilidades locomotoras.

Palavras-chave: predação larval; susceptibilidade à predação; desenvolvimento larval; Dendrobatoidea.

DEDICATÓRIA

Dedico este estudo a todos que se desafiam dia após dia a continuarem fazendo ciência, ainda mais no Brasil atual (principalmente nós que trabalhamos com Pesquisa de Base). Ser brasileiro é ser resistência. Ser brasileira, mulher, cientista e artista é um desafio que escolho todos os dias porque sei que, de alguma forma, esse esforço mudará a vida de alguém como mudou a minha. Se há duas coisas que transformam vidas, estas são a ciência e a arte e, por sorte, faço parte de ambas.

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“Pai, não ‘tô na área que o senhor queria

Mãe, lembra que eu disse que conseguiria?

Minha vitória não é saber que eu tenho grana

E sim reconhecer que sem Deus não aconteceria

Se precisasse, eu

Faria tudo de novo”

(GRATIDÃO - L7NNON)

RESUMO

Durante o desenvolvimento, larvas de anfíbios (girinos) passam por mudanças que alteram suas habilidades individuais. Enquanto espera-se que as habilidades sensoriais e cognitivas se desenvolvam progressivamente, as habilidades locomotoras podem ser inferiores tanto em estágios iniciais quanto finais. Desse modo, o desenvolvimento da capacidade de fuga pode ser dependente de habilidades desacopladas nestes organismos. Neste estudo, avaliamos experimentalmente como a vulnerabilidade à predação variou ao longo da ontogenia de girinos de rãs cuidadoras frente a larvas de baratas d'água como predadores. Com base nas habilidades sensoriais e cognitivas, testamos a hipótese de que as fases mais vulneráveis são as iniciais, esperando uma relação linear. Com base nas habilidades locomotoras, testamos a hipótese de que as fases mais vulneráveis são as iniciais e tardias, esperando uma relação quadrática. Para tanto, pareamos 105 girinos com potenciais predadores considerando os estágios de desenvolvimento e medidas morfométricas dos girinos, bem como variáveis-resposta relacionadas à latência e quantidade de ataques sofridos. A vulnerabilidade a ataques é menor em girinos em estágios avançados, durante os quais as vantagens sensoriais e cognitivas devem ser preponderantes. Interessantemente, a tendência de relação quadrática também foi observada, indicando que estágios avançados também sofrem de desvantagens locomotoras. Nenhuma das variáveis-preditoras mostrou efeito sobre a latência, sugerindo que a decisão de quando atacar e insistência na captura dependem majoritariamente do predador. Todas as variáveis morfológicas foram altamente correlacionadas, indicando que podem ser equivalentes. Contudo, o comprimento total do girino e comprimento da cauda melhor descrevem a vulnerabilidade a ataques.

Palavras-chave: *Allobates sumtuosus*, Amazônia, Aromobatidae, Dendrobatidae, desenvolvimento ontogenético, interações predador-presa, susceptibilidade à predação, desenvolvimento larval.

ABSTRACT

During development, amphibian larvae (tadpoles) go through changes that modify their individual abilities. While sensory and cognitive abilities are predicted to develop progressively, the locomotor abilities would be lower in initial and later stages. Thus, the development of escape ability can depend on decoupled skills in these organisms. Here we experimentally investigated how vulnerability to predation varies throughout tadpole ontogeny in nurse frogs facing giant water bug larvae as predators. Based on sensory and cognitive abilities, we tested the hypothesis that the most vulnerable phases are the initial ones, expecting a linear relationship. Based on locomotor skills, we tested the hypothesis that the most vulnerable phases are the early and late ones, thus expecting a quadratic relationship. For that, we paired 105 tadpoles with potential predators considering the developmental stages and morphometric measures of the tadpoles, as well as response variables related to latency and number of predator attacks. The vulnerability to predation is lower in advanced stages, during which the sensory and cognitive advantages should play a predominant role. Interestingly, a quadratic relationship trend was also observed, indicating that late larval stages also suffer from locomotor disadvantages. None of predictor variables affected latency, suggesting that the decision to attack and the insistence on capture depend largely on the predator. All morphological variables were highly correlated, indicating that they can be equivalent. However, total tadpole length and length of the tail better describe attack vulnerability.

Key-words: *Allobates sumtuosus*, Amazonia, Aromobatidae, Dendrobatidae, larval development, ontogenetic development, predation susceptibility, predator-prey interactions.

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INTRODUÇÃO GERAL

Predação consiste em uma interação entre indivíduos na qual um deles mata e/ou consome parte significativa da biomassa de um heteroespecífico (Abrams 2000). Portanto, os efeitos diretos dessa interação podem ser letais ou não letais. Efeitos letais influenciam a estrutura e tamanho das populações de presas através do consumo e consequente morte dos indivíduos (Lima e Dill 1990). Efeitos não letais, seja por injúria ou mera presença do predador, possibilitam alterações no comportamento da presa (Abrams 2000), o que pode afetar sua detecção e captura posteriores (Lima 1998; Abrams 2000; Abjörnsson, Brönmark e Hasson et al. 2002). Ambos os efeitos exercem pressão na seleção de mecanismos antipredação das espécies, sendo que diferentes predadores geram diferentes pressões (Hero, Gascon e Magnusson 1998).

Os mecanismos antipredatórios podem ser divididos em defesas primárias, secundárias ou mistas (Edmunds 1974). Em anfíbios, a exibição dos mecanismos primários independe da presença do predador, mas influencia no potencial de encontro entre predador e presa, como o aposematismo e a cripticidade (Eterovick 2000; Nomura et al. 2011; Guimarães et al. 2021). Os mecanismos secundários são exibidos após a detecção ou ataque do predador, tais como redução de atividade (e.g. Relyea 2003), retaliação (e.g. Wildy et al. 2001) e fuga (e.g. Wells 2007; Hemnani et al. 2022). O mecanismo misto pode funcionar tanto na prevenção do encontro quanto na detecção e defesa ao ataque do predador, como as agregações defensivas de cardumes (e.g. Spieler 2005). Esses mecanismos dependem das características morfológicas, fisiológicas e experiência da presa (Urszán et al. 2015), as quais parecem variar entre táxons (Toledo, Sazima e Haddad 2011) e durante a ontogenia das espécies, embora revisões sistemáticas sobre o tema, incluindo fases larvais, não tenham sido realizados até o momento (Ferrante et al. 2022).

A ontogenia se refere ao processo de desenvolvimento do indivíduo, que em anfíbios vai desde a fase de ovo fertilizado até a adulta (Garcia e Fernández 2012). Ao longo do seu ciclo de vida um indivíduo pode responder de diferentes maneiras a um mesmo estímulo devido às mudanças morfofisiológicas que afetam a maneira destes explorarem seu habitat (e.g. Lang et al. 1977). Isso pode promover mudanças em suas habilidades motoras (e.g. natação em girinos Wassersug e Sperry 1977; Wells 2007), cognitivas, sensoriais (e.g. *Bufo scaber* Gramapurohit et al. 2006), respiratórias (respiração branquial externa para pulmonar e cutânea Gosner 1960) entre outras que podem reduzir ou aumentar o risco de predação. Estas habilidades são fundamentais para a coexistência de girinos e sua grande variedade de predadores (Azevedo-Ramos, Magnusson e Bayliss 1999; Hero et al. 2001), tanto vertebrados (principalmente peixes) quanto invertebrados (e.g. larvas de Odonata, Heteroptera, Hemiptera). Sabe-se que girinos mais jovens, com menor tamanho e com caudas menores têm menor capacidade natatória (Wassersug e Sperry 1977; Wells 2007; Cheron et al. 2021), que a presença de membros posteriores pode influenciar negativamente no hidrodinamismo (Cheron et al. 2021), que durante a ontogenia passam por mudanças respiratórias onde há transição da estrutura branquial, de brânquias externas (estágios 19 a 24) para internas (estágios 25 a 42) de (Gosner 1960), mudanças na estrutura do aparato bucal que já no estágio 41 atrofiam para passar pelo período de metamorfose (Gosner 1960). Período esse que é propositalmente curto para que os anuros não permaneçam tanto tempo nessa forma mais vulnerável (Wassersug e Sperry 1977) onde não é capaz de se alimentar (Hu et al. 2022) e nem de se fugir com eficácia dos predadores, uma provável consequência direta do formato corporal não ser propício à locomoção eficaz (Wassersug e Sperry 1977). Sendo assim, tanto os estágios iniciais quanto os pré-metamórficos e metamórficos poderiam ser considerados mais suscetíveis à predação.

O conhecimento da relação entre desenvolvimento dos girinos e a natureza dos ataques por predador pode revelar informações importantes sobre mudanças na vulnerabilidade à predação durante a ontogenia, como já foi testado em lagostas (Lang et al. 1977). Contudo, a maioria dos estudos com larvas de anuros agrupam os estágios de Gosner (1960) em categorias maiores, geralmente sem justificativa biológica para agrupá-las (e.g. estágios 25-27, 28-30, 31-38 Wilson e Franklin 2000) ou considera estágios pontuais (e.g. estágios 25 e 42 Kuan e Lin 2011; estágios 42 a 46 Fabrezi et al. 2014; estágio 25 Achtymichuk et al. 2022). Isso também se reflete em outras áreas de pesquisa que utilizam girinos como objeto de estudo, como na diferença de produção de substâncias (Hayes et al. 2009), na tolerância ao calor (Agudelo-Cantero e Navas 2019) e preferência social (Hase e Kutsukake 2019). Até onde é sabido, não há estudos que abranjam a maioria dos estágios de Gosner (1960) quanto à susceptibilidade à predação. Os estudos disponíveis concentram-se em determinados estágios de desenvolvimento larval, o que impede um entendimento amplo acerca do tema, bem como comparações entre diferentes investigações.

Dessa forma, nosso objetivo foi entender como a vulnerabilidade à predação varia ao longo do desenvolvimento de larvas de anuros, avaliando tanto do ponto de vista do crescimento quanto do desenvolvimento destas potenciais presas. Para tanto, utilizamos como sistema modelo, a interação das larvas da rã-cuidadora *Allobates sumtuosus* (Morales 2002) como presa e larvas de barata d’água *Belostoma stollii* (Amyot & Serville, 1843) como predador de emboscada, orientado pela visão e movimentação (Victor e Ugwoke 1987), expostos em um experimento de encontro predador presa (primeira fase da predação Litvak e Leggett 1992), onde o predador não tem permissão de escolha da presa.

OBJETIVOS

Geral: Investigar como a vulnerabilidade à predação varia ao longo do desenvolvimento ontogenético de girinos;

Específico 1: Investigar se os estágios de Gosner (1960) podem ser substituídos por variáveis morfológicas contínuas de crescimento para descrever as fases ontogenéticas de girinos;

Específico 2: Avaliar se os girinos são mais vulneráveis somente nas fases iniciais ou nas fases iniciais e tardias de desenvolvimento.

CAPÍTULO ÚNICO

Dutra-Nunes, M.R.M.; Kaefer, I.L. & Cornelius, C. **How does vulnerability to predation vary throughout tadpole ontogeny?** Manuscrito submetido para *Behavioral Ecology* (A1 - Classificação de Periódicos Quadriênio 2017-2020 da CAPES.

How does vulnerability to predation vary throughout tadpole ontogeny?

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INTRODUCTION

The likelihood of an individual being consumed by a predator often varies throughout their lifetime. However, how this occurs is still poorly understood, especially in organisms that have a free-living larval stage. Survival during larval development is guided by several hypotheses that have been contested over time, especially in the areas of fishery resources (e.g., “Critical period hypothesis” Hjort 1914; “Match-mismatch hypothesis” Cushing 1975). By adding mortality and growth effects, predation gained a crucial role in the question of survival and hence the complementary hypotheses “the bigger the better” (Miller et al. 1988) and “larval stage duration” (Houde 1987). The first claims that larger, well-fed larvae are able to avoid predation more effectively (Neilson et al. 1986; Miller et al. 1988; Morote et al. 2010) because they have better swimming abilities associated with morphological development (Miller et al. 1988; Chick & Van Den Avyle 2000; Faria, Chícharo & Gonçalves 2011a). The second predicts that the early stages of development are more vulnerable to predation (Hare & Cowen 1997).

Both hypotheses have already been challenged in studies involving several animal groups (e.g., larvae of Odonata, Norma-Rashid 1999; juvenile lemon sharks, Dibattista et al. 2006; wolves, MacNulty et al. 2009). For fish larvae, mostly the larger larvae are more often attacked due to their size as they are easier to be detected by visual predators (Litvak & Legget 1992), and probably represent a more desirable prey to the predator, according to the theory of optimal foraging, where predators will consume prey selectively, in order to optimize the cost-benefit of the effort required to obtain the resource (Stephens & Krebs 1986). However, during larval ontogeny, in addition to increased growth (size), there are also developmental phase changes that potentially affect susceptibility to predation (Kuan & Lin 2011). However, the rate of development is naturally more accelerated than the rate of growth (Kuan & Lin 2011). In this way, morphological changes can alter the vulnerability to predation not only among the groups of species studied, but also according to the ontogenetic phase of the individual.

Among the morphological differences in the development of anuran larvae, it is known that tadpoles undergo changes according to morphological patterns, most of them grouped into 46 stages from the classic study by Gosner (1960). However, there are other classifications used to simplify the characterizations according to the questions of the different studies. As examples, we have classifications based on less refined stages such as eggs, newly hatched, swimming and premetamorphic (Azevedo-Ramos, Magnusson & Bayliss 1999); early, midstage and late stages (Hayes et al. 2009); premetamorphic, pro-metamorphic and metamorphic climax (Hu et al. 2022), among others. Such classifications can be directly associated with their developmental stages, from locomotor performance to the physiology of organs that interfere with how they can explore the environment (e.g., tail resorption, gill resorption and change to cutaneous respiration, shortening of the intestine, food reduction by change in the oral apparatus (Brown & Cai 2007; Fraser et al. 2020; Hu et al. 2022). Cheron et al. (2021) state that these changes are not linear throughout development. In this way, there would be two main behavioral phases: the first with an increase in activity and locomotion with the growth of the tail, followed by a phase of reduced locomotion by the resorption of the tail and other morphological and behavioral changes associated with metamorphosis that can make tadpoles more vulnerable again until the end of the larval phase.

Knowledge of the relationship between tadpole development and the nature of predator attacks can reveal important information about changes in vulnerability to predation during ontogeny. However, most studies with anuran larvae tend to group Gosner’s (1960) stages into more inclusive categories, usually without biological justification for this grouping (e.g., stages 25-27, 28-30, 31-38, Wilson & Franklin 2000) or consider only specific stages (e.g., stages 25 and 42, Kuan & Lin 2011; stages 42 to 46, Fabrezi et al. 2014; stage 25, Achtymichuk et al. 2022). As far as we know, no study has comprehensively investigated most of Gosner's (1960) stages regarding vulnerability to predation, thus neglecting the ontogenetic development of anuran larvae; a methodological decision that may eventually prevent a broader understanding of the topic, as well as comparisons between different studies.

Thus, our objective was to evaluate how vulnerability to predation varies throughout the development of anuran larvae, both from the point of view of growth and from that of development. Therefore, as a model system, we used the interaction of the larvae of *Allobates sumtuosus* (Morales 2002) as prey and the water bug larvae of *Belostoma stollii* (Amyot & Serville, 1843) as the ambush predator, which are guided by vision and movement (Victor & Ugwoke 1987). These were exposed in a predator prey encounter experiment (first phase of predation, Litvak & Leggett 1992), where the predator is not allowed a choice of prey.

We first evaluated whether Gosner's discrete stages (1960) and continuous morphological growth variables could be used as substitutes for each other to describe the ontogenetic phase of tadpoles, and our hypothesis is that the morphological growth variables would not be directly related to the ontogenetic stages, since the rate of development is more accelerated than that of growth and these determine complementary parts of ontogeny (Kuan & Lin 2011). Thus, the prediction would be that there would not necessarily be a correlation between growth and development variables in these organisms.

Armed with the premises of which variables would best explain the ontogeny of tadpoles, we investigated how vulnerability to predation varies throughout the development of *A. sumtuosus*, and our hypotheses were that the vulnerability of tadpoles (1) would decrease in a linear relationship, whereby younger tadpoles would be more vulnerable, or alternatively, (2) would decrease with development and increase again near metamorphosis, forming a quadratic relationship. The most vulnerable tadpoles would suffer attacks and captures more rapidly, as well as a lower number of attacks until the end of the experiment. In this study, a lower number of attacks suffered by the tadpole indicates that its antipredation defense or escape capacity, was not efficient. In turn, a greater number of attacks suffered indicates greater resistance of the individual and, consequently, lower susceptibility to death by predation.

METHODS

Place of study. The experiment was conducted in a laboratory environment located on the Experimental Farm of the Federal University of Amazonas - FAEXP (2°38'57.6"S, 60°3'11"W, Manaus, Brazil). The local vegetation is classified as Tropical Humid, *terra firme* forest, with closed canopy and understory with low luminosity (Guillaumet & Kahn, 1982; Almeida et al. 2014). The annual rainfall is 2,362 mm during the rainy season (November to May) (Marques-Filho et al. 1981; Araújo et al. 2002), with low variation in air temperature throughout the year, ranging between 24.6 ºC and 26.9 ºC (Araújo et al. 2002).

The collection of tadpoles and predators and the experiment were conducted between March and May 2021, during the rainy season (Marques-Filho et al. 1981; Araújo et al. 2002) and was concomitant with the reproduction period of most anurans, including the model species *Allobates sumtuosus* (Lima et al. 2012). The collection was carried out in temporary pools of water located in *terra firme* forest with the help of sieves and spoons. The specimens were immediately transported to the laboratory environment located at FAEXP.

Laboratory maintenance. The tadpoles were placed in basins with water from the pools from which they were collected and then transferred to basins with water from the fish tank. Post filtration of larger particles was performed with the aid of a sieve. The tadpoles were then screened to ensure they were of the same species. In the basins where the tadpoles were kept, we placed leaves from the collection site that serve as food and a refuge for the tadpoles before they were selected for the experiment. Tadpoles that were not of the desired species were separated to serve as food for the larvae of *B. stollii* during their stay in the laboratory. The *B. stollii* larvae were separated in 200 ml plastic cups half-filled with water (100 ml) to avoid cannibalism and escape from the cups.

Preparation of specimens for the experiment (pairing). The total length in millimeters of the *B. stollii* was measured with a digital caliper, and their weight in grams was measured with a 0.1 g precision scale, and photographs were taken with a 50 mm scale in the upper view. The tadpoles were randomly selected, separated into glasses with water (50 ml) to facilitate future pairing with the predator. Tadpoles were measured in grams using a 0.1 g precision scale, total length was measured with a digital caliper and then photographs were taken in top view with a 50 mm scale. From the total length values, we performed a pairing so that the prey was always smaller than the predator (the size ratio between total length of the predator and total length of the prey varied between 1.0009 and 1.991 mm), to minimize the effect of the size difference between predator and prey. After pairing, the tadpoles went through the verification phase of the stage, defined with the help of a stereomicroscope consulting Gosner’s table (1960). After having their stage determined, the tadpoles were exposed to the predator in the first phase of the experiment. The predators underwent a fasting period before the experiment (16-22 hours). The entire phase of preparation for the experiment was carried out by the same researcher.

Design of the experiment. The experimental arena consisted of a cube-shaped aquarium (9 x 9 x 9 cm), with 250 ml of water, pH 6.8, a temperature of 25 ºC, and indirect white artificial lighting.

The experiment consisted of three phases: acclimation of prey, pre-predator and with predator. Phase (1) was to place the tadpole in the center of the arena and leave it alone for 10 minutes for acclimation. After this period, the pre-predator phase (2) began in which the tadpole was recorded for 20 minutes alone in the arena. After the end of phase 2, phase (3) began with the insertion of the predator (*B. stollii*) with the help of a spoon at the opposite end of the aquarium, as far as possible from the place where the tadpole was. This phase lasted for 20 minutes.

The recording was performed with a video camera (Canon, SX530 HS) at an angle of 90º, for a total of 50 minutes. No individual predator or prey was used more than once in order to avoid a possible learning effect. All experiments were performed during the daytime period (between 8:00 and 16:00), in which the larvae of *Belostoma stollii* are most active (personal obs.).

Post-experiment procedures. For the purpose of refining the variables collected for the pairing, the images of the tadpoles and *B. stollii* larvae were evaluated using the Image J software to acquire the morphological variables of growth in millimeters, respectively: total length, tail length and height of the caudal muscle of the tadpoles, and total length of the *B. stollii* larvae.

All *B. stollii* were fixed in 70% alcohol, which is standard procedure for most invertebrates (e.g., Rudolf & Eveland 2021). All surviving tadpoles were euthanized with an overdose of lidocaine gel according to the guidelines of the National Council for Animal Control and Experimentation (CONCEA) and fixed in 5% formalin (e.g., Menin & Giaretta 2003; Menin, Lima & Rodrigues 2010). The tadpoles were deposited in the Prof. Paulo Bührnheim Zoological Collection at the Federal University of Amazonas (CZPB-UFAM) and water bugs were deposited in the Invertebrates Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

We opted for euthanasia and fixation of the tadpoles involved in the experiments for the following reasons: (1) in nature, tadpoles have high mortality rates, which can reach 95% up until metamorphosis (Semlitisch 1987; Semlitisch et al. 1996). Therefore, reintroducing tadpoles at more developed stages (Claytor et al. 2017) could affect the survival and performance of larvae, thus generating local overpopulation and, consequently, trophic changes in a cascade effect (Wissinger et al. 1999) in both aquatic and terrestrial environments, since the resources consumed by amphibian larvae are transferred to terrestrial habitats after metamorphosis (Gibbons et al. 2006); (2) *A. sumtuosus* is widely distributed, relatively abundant and does not appear on any threatened list, so any impact on local population would not significantly affect the conservation of the species or its lineages; (3) the density of tadpoles under experimental conditions could facilitate the transmission of pathogens, whose dispersion via reintroduction into the wild should be avoided (Pavanelli et al. 2000; Brunner et al. 2015).

Ethical considerations. This research was approved by SISBio (license No. 760601) and by the Ethics Committee on the Use of Animals of the Federal University of Amazonas under No. 019/2020/CEUA.

We chose to identify the stages (Gosner 1960) and measure the total length of the specimens with a digital caliper for the pairings without anesthesia. Always on the same day that predator and prey would be subjected to the experiments. This decision was made because during the experiment the prey could suffer non-lethal or lethal attacks (Abrams 2000), which would make it impossible to verify the development and growth variables used in this study. Additionally, the effect of anesthetics could influence several functions in the body beyond the nervous system, thus interfering with the results of the behavioral research (Coppo, Mussart & Fioranelli 2002; Achtymichuk et al. 2022).

Data analysis. A total of 105 predator-prey encounter experiments were performed, and we used 20 minutes of the recording of phase 3 of the experiment, which involved the interaction between predator and prey. To quantify the vulnerability of the tadpoles, we considered three operational variables: number of attacks, latency in seconds for the first attack and latency in seconds until the final attack (capture). All analyses of the recordings were performed by a single researcher. Attack was recorded whenever the *B. stollii* larvae approached the tadpole concomitantly with the closure of its front raptorial paws (Victor & Ugwoke 1987).

Correlation among predictor variables. To access the equivalence between Gosner's (1960) stages and continuous morphological variables, we used Pearson's correlation analysis. We considered the variables as being correlated when the value of $r > 0.7$.

Variation of number of attacks and ontogeny variables. We analyzed the relationship between the number of attacks and ontogeny via generalized linear models (GLMs) with Poisson distribution. The models were performed with the ontogenetic variables as predictors: Gosner stages as a development variable and morphometric variables (total length, tail length and caudal muscle height) as growth variables.

To determine which type of relationship (linear or quadratic) best explained the variation in the number of attacks, we included a linear and quadratic model for each predictor variable and employed the model selection approach based on the Akaike information criterion (Burnham & Anderson 2002). In this approach, AICc is the numerical value indicative of the amount of information from the model, ΔAIC is the result of the difference between the AICc of the best model and the model in question, df refers to the degrees of freedom/number of parameters used in the model, and *w*i is the weight of the model (Burnham & Anderson 2002).

We considered a set of three models including a constant model (null), the linear model and the quadratic model for each predictor variable (Table 1), and each model represents a possible hypothesis about the source of variation of the response variable (number of attacks). Then, we compared all the models, contrasting the effect of all predictor variables on the number of attacks, totaling nine models (one for each predictor variable, with linear or quadratic response, as well as a constant model) to determine which of the predictor variables has a greater strength of evidence to predict the number of attacks (Table 2). Weight values (*w*i) were used as evidence of the measure of strength of each model and were compared between the selected models (with ΔAIC < 2). The AIC analysis was performed with the *bbmle* package in R software, version 3.6. (R-Core-Team 2018). At the end, we estimated the parameters for the two best models, which were selected for comparative purposes (Table 3).

Variation of time and latency. To analyze these metrics, we opted for the failure analysis approach (Fox 2001) in which we recorded the latency times until the first attack and until the definitive attack (capture) in the period of 20 minutes (1,200 s). This approach is characterized by quantifying the time until a certain event happens. When the attack occurs, it is considered a failure and the time elapsed until that event is called survival time (Fox 2001). In the case of our experiment, a failure event was when the tadpole suffered the first attack or suffered the definitive attack. The failure could only occur once for each latency.

A hazard ratio is given based on the chance of a failure occurring at a given time. To evaluate how time influences the risk rate, a survival model with a distribution defined by a parameter in the form of (p) is tested (Fox 2001). When $p < 1$, the chance of failure risk decreases as a function of time. For example, the more time that passes for the first attack to occur, the less chance of this attack happening. However, if $p > 1$, the opposite is true. When

$p \neq 1$, the distribution is called Weibull, and when p = 1, the distribution is called exponential. If $p = 1$, the risk rate is considered constant, in other words, the chance of the event happening is the same regardless of the observation time. If the given event was never observed during the observation period (for example, a tadpole that never suffered attacks during the experiment or never suffered capture), the data is considered censored (Fox 2001). In this way, the distribution refers to the type of distribution known as the failure analysis (exponential or Weibull), with the *p*-value (hazard ratio) representing the influence of time on the event being analyzed.

We used the latency times in the models as a response variable (time until the first attack and time until definitive attack) using the *survreg* function in the R software, version 3.6.2 (R-Core Team 2018) and the ontogeny variables as predictors (Gosner stages, total length, tail length and caudal muscle height). We modelled the hazard ratio with the Weibull and exponential distributions, which led to 10 models for each variable latency time response (latency to first attack and latency to definitive attack): (1) ~ Gosner stage (Weibull), (2) ~ Gosner stage (exponential); (3) ~ tadpole total length (Weibull); (4) ~ tadpole total length (exponential); (5) ~ tadpole tail length (Weibull); (6) ~ tadpole tail length (Weibull). (exponential); (7) ~ caudal muscle height (Weibull); (8) ~ caudal muscle height (exponential); (9) ~ 1 (Weibull) and (10) ~1 (exponential).

RESULTS

At the end of the experiments, 105 predator-prey pairs had been tested. Of these, 76.2% of the tadpoles had suffered at least one attack. Continuous predicting variables showed the following results: the total length of the tadpoles varied from 9.71 mm to 23.22 mm (16.5 ± 3.04 mean ± SD), the caudal length varied from 4.24 mm to 14.59 mm (9.988 ±

2.179) and the height of the caudal muscle varied from 0.53 mm to 2.15 mm (1.304 ± 0.3288).

The response variables behaved as follows: the number of attacks ranged from 0 to 14 attacks (2.05 ± 2.48), the latency to the first attack ranged from 1 to 1,174 seconds (222.7 ± 333.4), the latency until the definitive attack ranged from 1 to 1,194 seconds (419.3 ± 396.1) and the difference between the first attack and the definitive attack ranged from 0 to 1,188 seconds (250.3 ± 356.6).

Correlation between predictor variables. All predictor variables were correlated with each other (r > 0.72) indicating a strong equivalence between them (Figure 1).

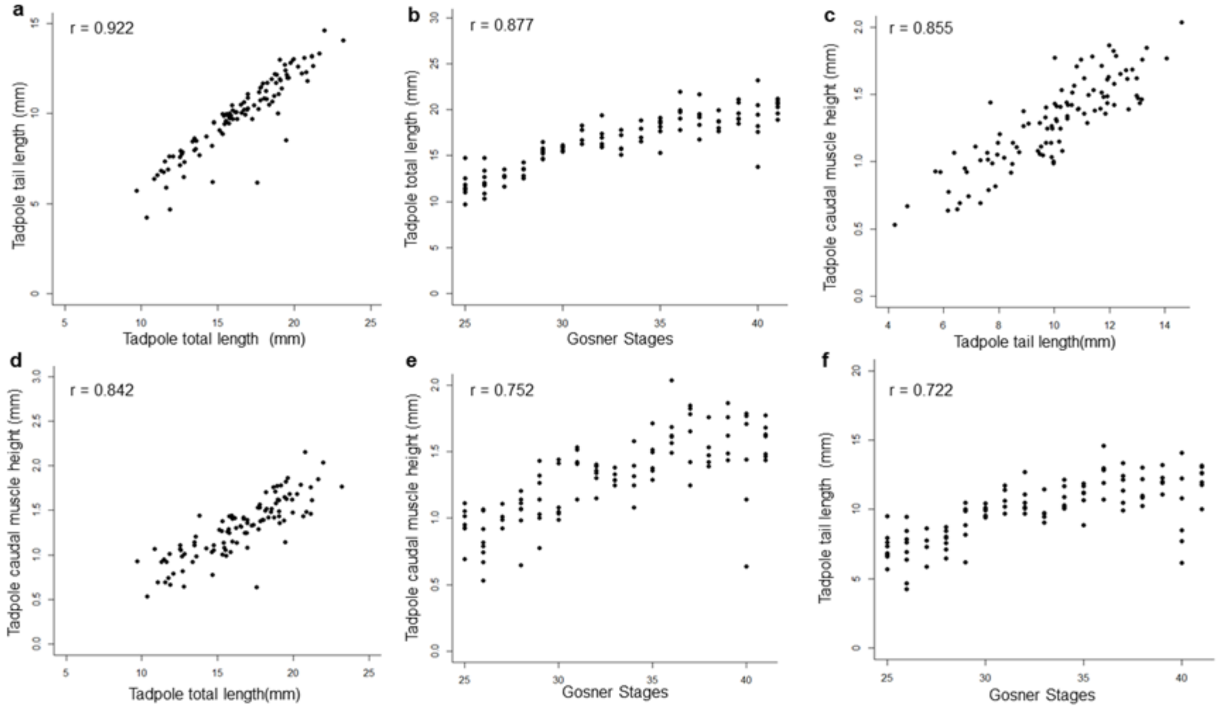


Figure 1. Predictor variables in descending order of correlation coefficient: (A) total tadpole length – tadpole tail length (r = 0.922), (B) Gosner stages – total tadpole length (r = 0.877), (C) tadpole tail length – tadpole caudal muscle height (r = 0.855), (D) total tadpole length –

tadpole caudal muscle height (r = 0.842), (E) Gosner stages – tadpole caudal muscle height (R = 0.752), and, (F) Gosner stages – tadpole tail length (R = 0.722).

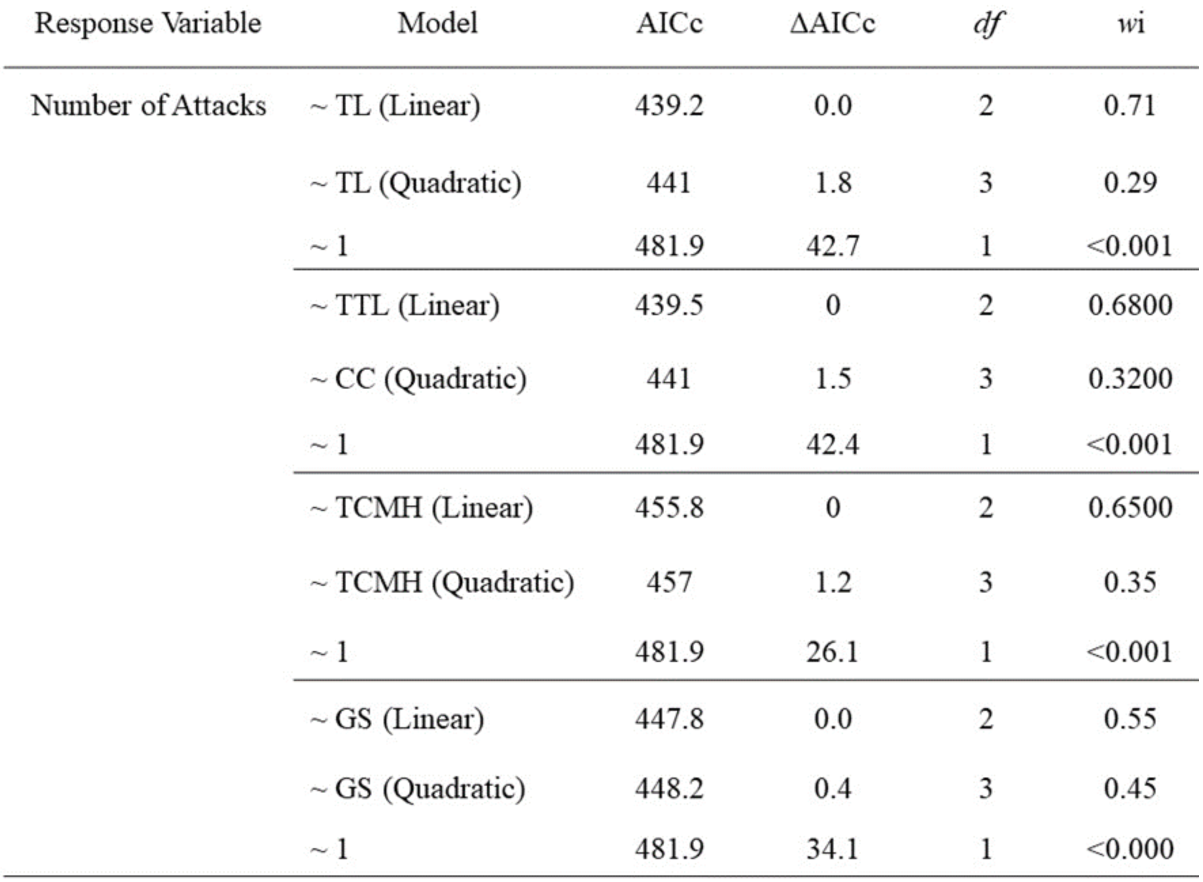
The strongest correlation was between tadpole total length and tail length (r = 0.92). The weakest correlation was between developmental stages and tail length (r = 0.72).

Behavioral analyses of response: attacks.

Analyses of the attack variable were performed with all 105 of the individuals, with the four morphological variables as predictors for the number of attacks.

Evaluating in isolation each set of three models of a type of predictor variable, in all cases, the linear model was the best (with weight *w*i, ranging from 0.55 to 0.71), followed by the quadratic model (with weight *w*i ranging from 0.29 to 0.45) and the constant, which was discarded in all cases (ΔAIC > 2).

Table 1. Variable-response model selection number of attacks as a function of the four predictor variables. TL – total length of the tadpole, TTL – length of the tail of the tadpole, TCMH – height of the caudal muscle of the tadpole and GS – Gosner's stage. The models with ΔAIC < 2 were selected as the best models to predict the relationship.

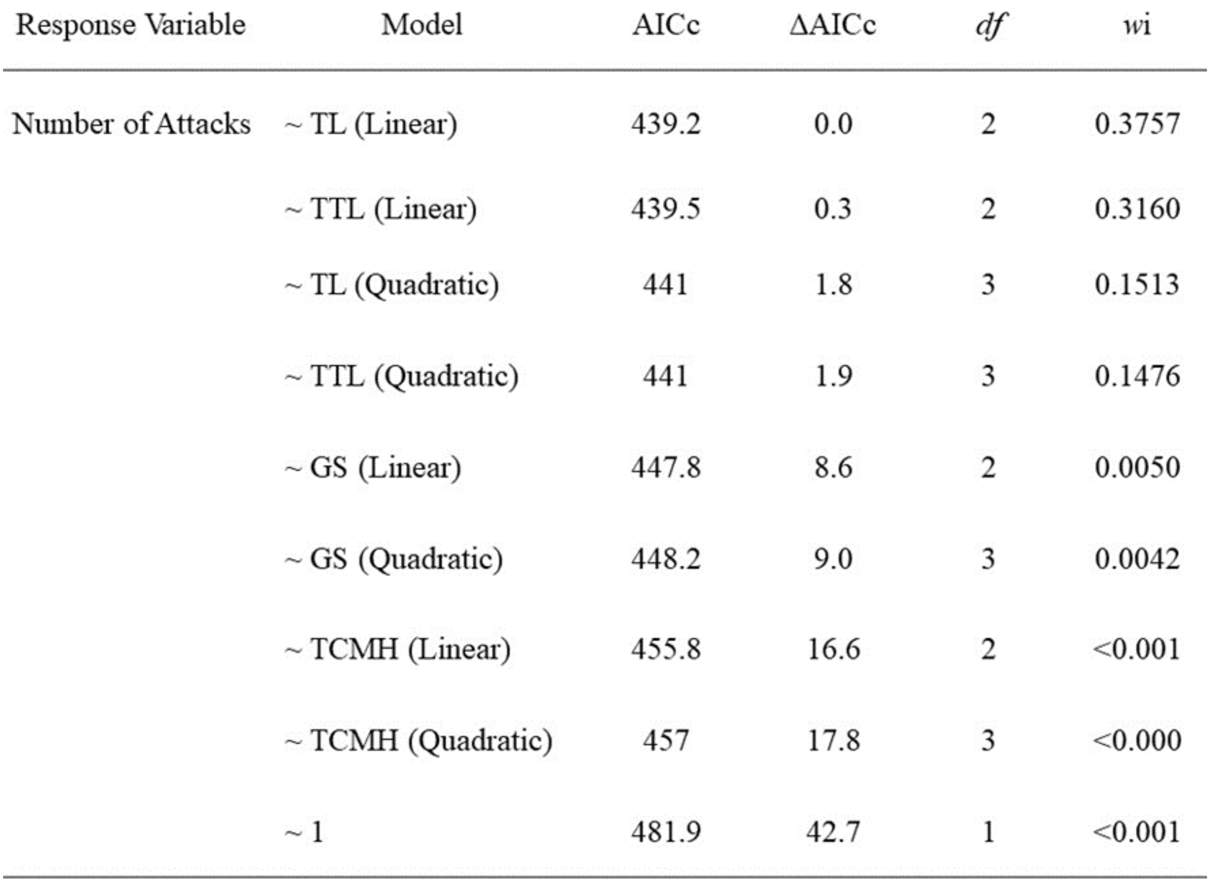


Response Variable	Model	AICc	ΔAICc	*df*	*wi*
Number of Attacks	~ TL (Linear)	439.2	0.0	2	0.71
	~ TL (Quadratic)	441	1.8	3	0.29
	~ 1	481.9	42.7	1	<0.001
	~ TTL (Linear)	439.5	0	2	0.6800
	~ CC (Quadratic)	441	1.5	3	0.3200
	~ 1	481.9	42.4	1	<0.001
	~ TCMH (Linear)	455.8	0	2	0.6500
	~ TCMH (Quadratic)	457	1.2	3	0.35
	~ 1	481.9	26.1	1	<0.001
	~ GS (Linear)	447.8	0.0	2	0.55
	~ GS (Quadratic)	448.2	0.4	3	0.45
	~ 1	481.9	34.1	1	<0.000

The non-inclusion of the constant model among the best models (with ΔAIC < 2) indicates that all variables in the selected models can be considered to explain the number of attacks. The superiority of the linear model indicates that the youngest and smallest tadpoles would receive the fewest attacks. The plausibility of the quadratic model (i.e., selected with ΔAIC < 2), even with lower strength of evidence, indicates a reduction in the number of attacks when tadpoles are larger. Additionally, only when using the stage variable, the linear and quadratic models could be considered equivalent since their parameters (AIC, ΔAIC and *wi*) were very similar (Table 1).

Table 2. Contrast of all models with the effect of each predictor variable on the response variable number of attacks and constant model. TL – total length of the tadpole, TTL – length of the tail of the tadpole, TCMH – height of the caudal muscle of the tadpole and GS –

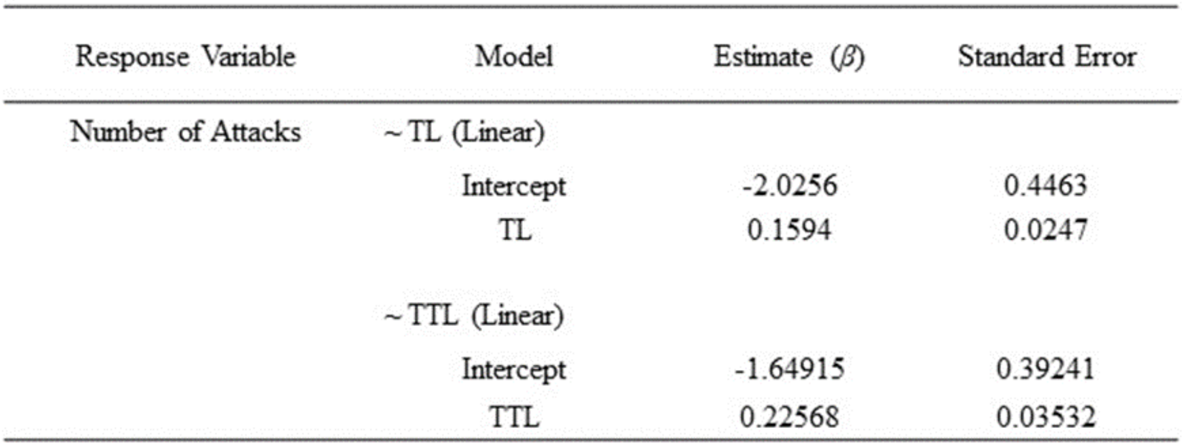
Gosner’s stage. The models with ΔAIC < 2 were selected as the best models to predict the relationship.



Response Variable	Model	AICc	ΔAICc	*df*	*wi*
Number of Attacks	~ TL (Linear)	439.2	0.0	2	0.3757
	~ TTL (Linear)	439.5	0.3	2	0.3160
	~ TL (Quadratic)	441	1.8	3	0.1513
	~ TTL (Quadratic)	441	1.9	3	0.1476
	~ GS (Linear)	447.8	8.6	2	0.0050
	~ GS (Quadratic)	448.2	9.0	3	0.0042
	~ TCMH (Linear)	455.8	16.6	2	<0.001
	~ TCMH (Quadratic)	457	17.8	3	<0.000
	~ 1	481.9	42.7	1	<0.001

After the contrasting of all the models, the variables with the best predictive power for number of attacks were tadpole total length and tail length (Table 2), which were also the variables that most correlated with each other (Figure 1 - A). These variables were selected in both the linear and quadratic models, totaling four selected models (ΔAIC < 2), but the models with the greatest strength of evidence were the linear models (Table 2). The possible quadratic relationship between the variables, evidenced by the models that ranked third and fourth, can be seen in the relationship of the attacks with the measurements of total length and tail length (Figure 2 - A, B). The other variables, stages and caudal muscle height, were not good predictors for the number of attacks (models with ΔAIC > 2).

Table 3. Parameters of the two best models selected to predict the number of attacks. TL - total length of the tadpole, TTL - length of the tail of the tadpole.



Response Variable	Model	Estimate (β)	Standard Error
Number of Attacks	~ TL (Linear)		
	Intercept	-2.0256	0.4463
	TL	0.1594	0.0247
	~ TTL (Linear)		
	Intercept	-1.64915	0.39241
	TTL	0.22568	0.03532

Larger tadpoles with longer tails received a greater number of attacks (Figure 2 A-B and Table 2), thus evidencing a positive linear relationship (Table 3), in which the probability of having a greater number of attacks increases with development and growth.

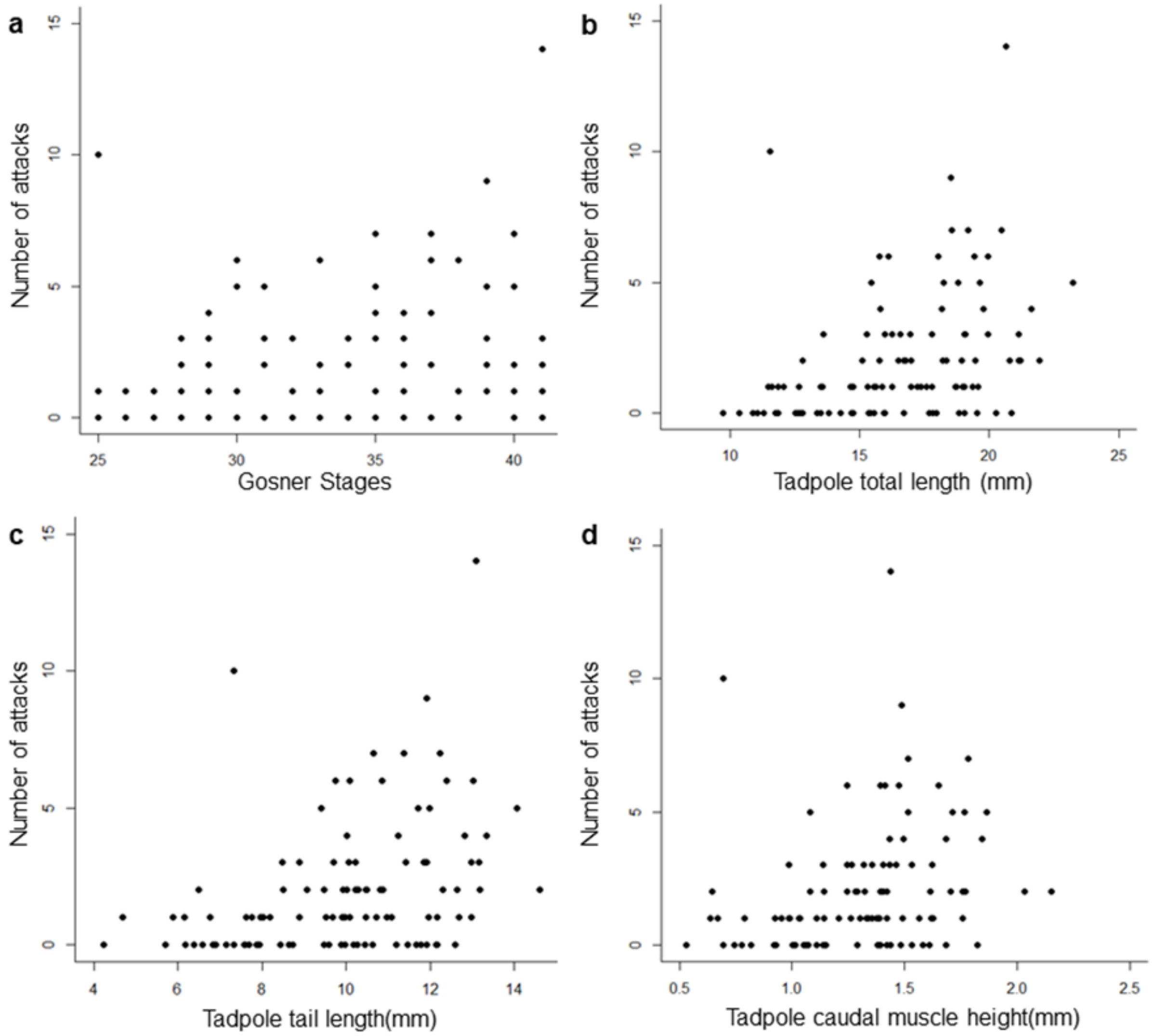


Figure 2. Generalized linear models between the number of attacks and: (A) total tadpole length, (B) tadpole tail length, (C) Gosner stages, and (D) tadpole caudal muscle height. In descending order, from the best model to the least plausible.

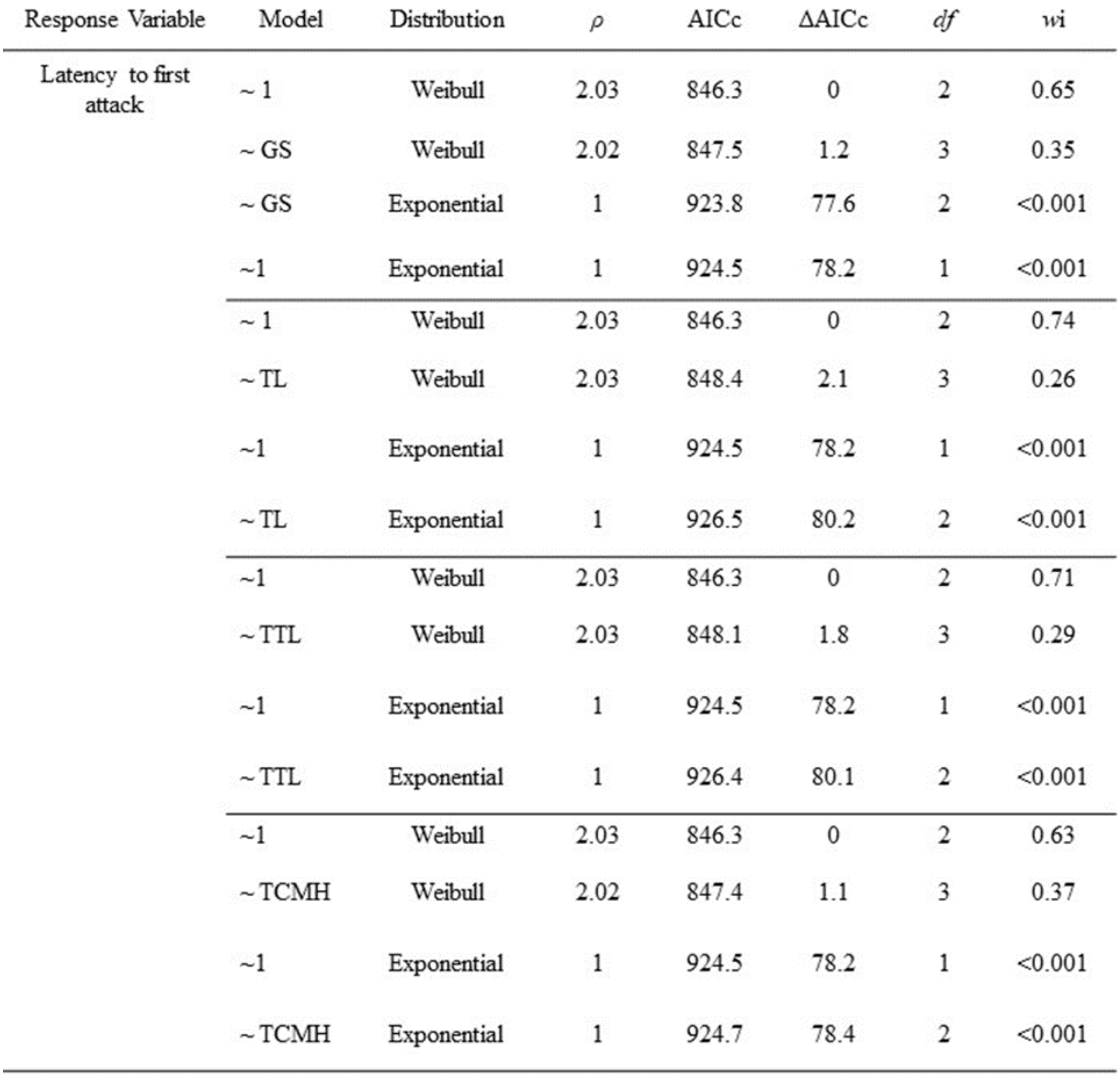
Tadpoles in more advanced stages and with a larger caudal muscle also received more attacks (Figure 2 C, D); however, the variables that best explain the number of attacks are the total length and tail length variables (Table 2 and Table 3).

Survival analyses: latency time

Latency to the first attack:

For all the morphological variables (of developmental and growth), the constant model with Weibull distribution ($p \neq 1$) was always selected as the most plausible to explain the latency to the first attack (Table 4).

Table 4. Variable-response model selection latency to first attack as a function of the four predictor variables. TL – total length of the tadpole, TTL – length of the tail of the tadpole, TCHM – height of the caudal muscle of the tadpole and GS – Gosner's stage. The models with ΔAIC < 2 were selected as the best models to predict the relationship.



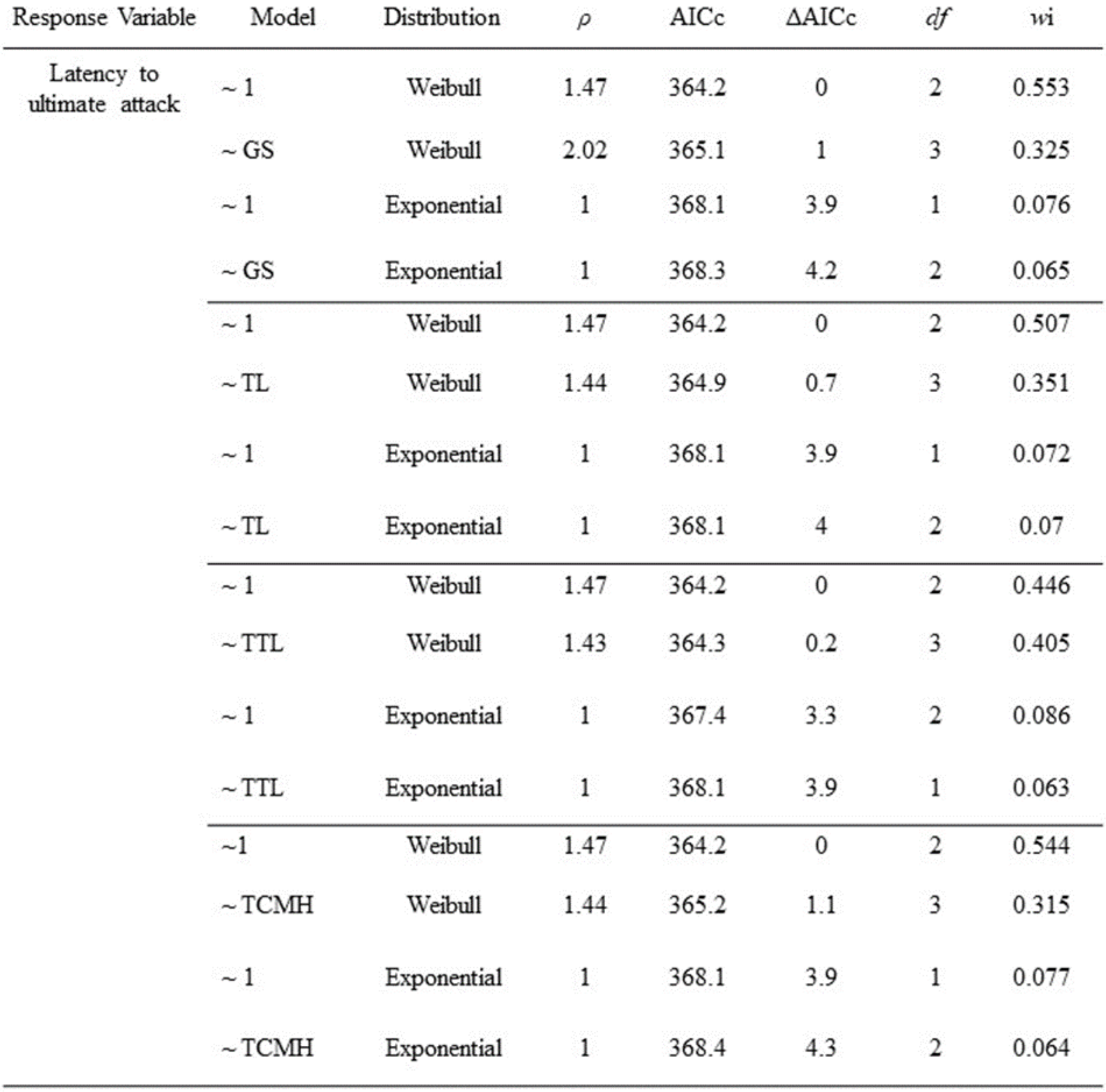
Response Variable	Model	Distribution	ρ	AICc	ΔAICc	*df*	*wi*
Latency to first attack	~ 1	Weibull	2.03	846.3	0	2	0.65
	~ GS	Weibull	2.02	847.5	1.2	3	0.35
	~ GS	Exponential	1	923.8	77.6	2	<0.001
	~1	Exponential	1	924.5	78.2	1	<0.001
	~ 1	Weibull	2.03	846.3	0	2	0.74
	~ TL	Weibull	2.03	848.4	2.1	3	0.26
	~1	Exponential	1	924.5	78.2	1	<0.001
	~ TL	Exponential	1	926.5	80.2	2	<0.001
	~1	Weibull	2.03	846.3	0	2	0.71
	~ TTL	Weibull	2.03	848.1	1.8	3	0.29
	~1	Exponential	1	924.5	78.2	1	<0.001
	~ TTL	Exponential	1	926.4	80.1	2	<0.001
	~1	Weibull	2.03	846.3	0	2	0.63
	~ TCMH	Weibull	2.02	847.4	1.1	3	0.37
	~1	Exponential	1	924.5	78.2	1	<0.001
	~ TCMH	Exponential	1	924.7	78.4	2	<0.001

In all cases, the weight of the best model (the constant model) is twice the weight of the models containing the predictor variables, which suggests that the latency time to the first attack is not being affected by any of the morphological variables used. However, the Weibull distribution (when *p* > 1) indicates that the chance of failure risk increases with time (Table 4).

Latency to definitive attack:

For all the morphological variables (growth and development), the constant model with Weibull distribution was also always selected as the most plausible to explain the latency time to definitive attack (Table 5), indicating that the morphological variables of the tadpole do not predict the time elapsed until the final attack. However, we note that the best model is also always the model with Weibull distribution, with p > 1 indicating that the chance of failure increases with time, which may indicate that the probability of attack increases with time. This is best seen in latency-to-capture results because the longer the predator and prey remained in the arena, the more likely prey was to be captured.

Table 5. Variable-response model selection latency to ultimate attack as a function of the four predictor variables. TL – total length of the tadpole, TTL – length of the tail of the tadpole, TCMH – height of the caudal muscle of the tadpole and GS – Gosner's stage. The models with ΔAIC < 2 were selected as the best models to predict the relationship.



Response Variable	Model	Distribution	*ρ*	AICc	ΔAICc	*df*	*wi*
Latency to ultimate attack	~ 1	Weibull	1.47	364.2	0	2	0.553
	~ GS	Weibull	2.02	365.1	1	3	0.325
	~ 1	Exponential	1	368.1	3.9	1	0.076
	~ GS	Exponential	1	368.3	4.2	2	0.065
	~ 1	Weibull	1.47	364.2	0	2	0.507
	~ TL	Weibull	1.44	364.9	0.7	3	0.351
	~ 1	Exponential	1	368.1	3.9	1	0.072
	~ TL	Exponential	1	368.1	4	2	0.07
	~ 1	Weibull	1.47	364.2	0	2	0.446
	~ TTL	Weibull	1.43	364.3	0.2	3	0.405
	~ 1	Exponential	1	367.4	3.3	2	0.086
	~ TTL	Exponential	1	368.1	3.9	1	0.063
	~1	Weibull	1.47	364.2	0	2	0.544
	~ TCMH	Weibull	1.44	365.2	1.1	3	0.315
	~ 1	Exponential	1	368.1	3.9	1	0.077
	~ TCMH	Exponential	1	368.4	4.3	2	0.064

The value of the shape parameter > 1 indicates a trend that the failure rate, i.e., the probability of an attack happening, will increase over time. All shape parameters are between $1 < p < 3.6$, which indicates an asymmetry on the right. Therefore, the longer the prey were exposed to the predator, the greater the possibility of attack and capture, however regardless of size or stage of development (Table 5).

DISCUSSION

Our results showed that vulnerability to predation decreases throughout the development and growth of *A. sumtuosus* tadpoles, and that the four growth and development variables evaluated (total tadpole length, tail length, caudal muscle height and developmental stage) evidence this relationship. However, two continuous morphological variables of growth (total length and tail length) better explained the variation in the number of attacks suffered by the tadpole than the height of the caudal muscle and Gosner's (1960) categorical variable of stages; the latter classically used in studies involving tadpoles.

Older and larger tadpoles were more frequently attacked, similar to what occurs in fish (Litvak & Legget, 1992). This may be because they possess greater escape ability attributed to greater sensory and cognitive abilities, making them harder prey to capture (Beck & Congdon 2000, Morey & Riznick 2001, Altweeg & Reyer 2003; Wells 2007; Kuan & Lin 2011) and are therefore less vulnerable to fatal attacks, similar to a more developed boxer who is harder to knock out. Alternatively, the observed result may be due to the preference of predators to insist on attacking larger prey, as predicted by the theory of optimal foraging, opting for the highest cost-benefit (Stephens & Krebs 1986).

The greater number of attacks suffered by larger larvae is consistent with the findings that tadpoles of greater total length (Wilson & Franklin 2000), of more advanced stages (Wassersug & Sperry 1977; Huey 1980; Wassersug & Hoff 1985; Brown & Taylor 1995; Parichy & Kaplan 1995) and with larger tails (Huey 1980) can reach greater swimming speeds (Wassersug & Hoff 1985; Wassersug 1989; Liu, Wassersug & Kawachi 1996; Wassersug 2000). This would give them greater escape capacity (Alton et al. 2012) and, therefore, would result in suffering a greater number of attacks if the predator insists on capturing these larger prey, particularly in spatially limited environments and without the availability of hiding places.

The tendency of increasing attacks in older tadpoles can be observed when we use Gosner's stages (1960) as a predictor variable, but it is with continuous growth variables that the relationship was stronger. This is because morphometric measurements of growth are more accurate and allow better inferences about ecological parameters (Norma-Rashid 1999); unlike categorical variables that artificially group individuals with distinct characteristics.

Although evidence of the progressive increase in the number of attacks in the later stages and in larger tadpoles was the most pronounced, a secondary model also showed a reduction in the number of attacks in larger tadpoles and in the later stages. This can be explained by the morphological changes that occur throughout stages 40 and 41 of development (Gosner 1960), in which the larvae usually reach their maximum body mass, cease growth and then begin a process of mass loss until the end of metamorphosis (Adolph 1931; Kuan & Lin 2011). At stage 41, the last considered in the present study, the tadpole is predicted to undergo a drastic reduction in locomotion capacity (Wassersug & Sperry 1977; Cheron et al. 2021). Such a reduction is characterized by the end of the development of the hind limbs (Gosner 1960), which are not yet useful for propulsion. Therefore, this condition must impair their performance in the fast-start swimming that is essential for escape from predators (Wilson & Franklin 2000), particularly in the experimental conditions of our study.

To better understand the decrease in the number of attacks in the later stages of pre-metamorphosis and metamorphosis, we encourage further studies that maintain tadpoles in this transition phase with a predator that is not strictly aquatic as in our experiment; since, from this moment on, the forelimbs are already developed (Gosner 1960) and the tadpole is capable of climbing up to terrestrial surfaces (personal obs.) via the inner walls of the experimental aquarium. With experiments of this type, it may be possible to test the predation vulnerability model suggested by Greene (1986), which suggests a dome-shaped curve generated by the product of the probabilities of attack and capture, which resembles our alternative hypothesis of the quadratic model.

Contrary to the hypothesis that we stipulated for the time elapsed for the first attack and time elapsed for the definitive attack to occur, these were not influenced by the morphology of the tadpole, and thus are independent of the characteristics of the prey in our study. This result indicates that the elapsed time for both types of attack has a greater relationship with individual characteristics of the predator (Victor & Ugwoke 1987), which affects their abilities to capture, handle and consume prey (Relyea 2001b), as well as their cost-benefit decision (Stephens and Krebs 1986) in relation to the attack. Since our experiment provided the “prey encounter”, which represents the first of the three sequential phases of the predation event (encounter, attack and capture, Litvak and Leggett 1992), the decision to attack (which influences the time until the first attack) and insistence on successive attacks for a possible capture (respectively, number of attacks and time until the definitive attack) seems to be predominantly that of the predator.

All the predictor variables tested were highly correlated with each other. Therefore, according to the question and the sample design of the study, using only one of them may be sufficient to refer to the “moment of life history” of the tadpole. However, we observed that continuous variables, especially total length and tail length, more accurately predicted the number of attacks that the prey will receive.

Our results indicate that, depending on the question investigated, stages can be used in isolation, without the need for tail length measurements and vice versa, which is an approach used in many studies involving tadpoles (e.g., categories of stages - total length, Hu et al. 2022; only stage 25, Achtymichuk et al. 2022). However, this equivalence should be considered with caution since, as we have shown here, continuous growth variables may be more accurate in describing certain relationships. Thus, we encourage the use and reinforce the advantage of using different classes of variables in order to compare studies that use different variables.

It is important to consider that since growth refers to size and development refers to age (Kuan & Lin 2011), and that both can be affected by ecophysiological factors, the rate of development can be decoupled from the rate of growth. For example, it is important that physiological studies, especially those involving changes in metabolic rates, consider all categories of variables concomitantly, since often the objective of these is precisely to understand the correlation between stages and morphometric variables in relation to environmental predictor variables (Atkinson, Helbing & Chen 1996). Tadpoles may undergo metamorphosis into different body sizes than what is expected for that stage due to the shortening or prolongation of the larval period (Newnman 1988, Relyea 2007; Walsh, Downie & Monaghan 2008; Hu et al. 2022) due to various stressors such as environmental contamination and climate change, among others.

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CONCLUSÕES

- Todas as variáveis preditoras de crescimento e desenvolvimento dos girinos são altamente correlacionadas, portanto a utilização de apenas uma delas pode ser possível de acordo com a questão científica abordada.
- A vulnerabilidade à predação diminui ao longo da ontogenia. Isso pode ocorrer devido a sua maior capacidade de fuga atribuída às maiores capacidades cognitivas, fisiológicas e locomotoras.

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