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LIFE-HISTORY AND BEHAVIOURAL ADAPTATIONS TO XERIC ENVIRONMENTS IN FOSSORIAL AMPHIBIANS

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Doctoral thesis summary

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Constanța, 2018

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ACKNOWLEDGEMENTS

First and foremost, I am hugely indebted to my promotors. Both of them have supplied equally invaluable, complementary contribution to my research. They generously provided their deep insight, extensive experience and most appreciated critiques, without ever restricting my freedom. This work is as much mine as it is theirs.

Many thanks to my guidance committee - Dragomir Coprean, Teodora Onciu and Marius Skolka for investing their time, experience and patience in my project. A particular mention to the one who focused my interest towards herpetology, Ioan Ghira. Even after so many years, he remains a main driving force of my research.

I was incredibly fortunate to benefit from interactions with several great specialists and receive help from so many wonderful people. I would like to thank the Romanian team – Florina Stănescu, Iosif Ruben, Elena Buhaciuc, Marian Tudor, Daniyar Memedemin, Raluca Băncilă, Rodica Plăiașu - along with making the field - and lab - work equal FUN, each of you have taught me so many lessons, that I will use forever.

I am grateful to the Ecuadorian team – Diego Armijos-Ojeda, Carlos Ivan Espinosa, Andrea Jara-Guerrero, Leonardo Ordóñez-Delgado, Rodrigo Cisneros, for constantly sharing their broad understanding of tropical ecosystems, along with providing support and guidance, and contributing to my deep love of Ecuador.

Many other researchers have contributed their experience and irreplaceable insights to many aspects of my studies - Ulrich Sinsch, Santiago R. Ron, Andrés Merino-Viteri, Cecilia Bardier, Rodrigo Cajade, and Martin Carlsson. Amongst them, a special place holds Federico Marangoni, who was the first to show me a Pacman and start it all.

Special thanks to the amazing "guardaparques" from Reserva Ecologica Arenillas, who made all the efforts to support my idiotic search for biting frogs. My fieldwork would have been impossible without the contribution of Carlos Domauri Tinoco, Andrez Chacha Bastidas, Lenin Ramon Jacome, Ángel Freire Díaz, Omar Aguilar Illescas, Luis Gainza Proaño, Rodolfo Arias Pintado, Gabriel Miranda Arias, Aldo Ceccone Buenaño, and Paul Medranda Rodriguez.

This project would not have been even imaginable without Paul. I cannot begin to express how grateful I am for this gift. My deepest gratitude to my parents, who have helped, supported and influenced me in countless ways.

The research received financial support from the Romanian National Authority for Scientific Research CNCS – UEFISCDI, through the grants PN-II-ID-PCE-2011-3-0173 "Is range limited by life history, environmental conditions, or biogeographical barriers? A study case using spadefoot toads (genus *Pelobates*)", PN-II-CAPACITĂȚI-732/2013 "The impact of global changes on populations at the limit of their ranges" and the Wallonie-Bruxelles International and CCCDI-UEFISCDI (ANCS) joint grant 105BM/2017 "Adaptive strategies in amphibians under environmental stress". Fieldwork was partially supported by the Prometeo Project of the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación, Ecuador (SENESCYT) granted to Dan Cogălniceanu and Paul Székely. Necessary permits were issued by Ministerio del Ambiente de Ecuador (research permit number MAE-DNB-CM-2015-0016) and UTPL Ethics Committee (ethical permit UTPL-CBEA-2016-001).

KEYWORDS

Aridity; burrowing; *Ceratophrys*; life cycle; life-history traits; mate choice; metamorphosis; phenotypic plasticity; sexual selection.

INTRODUCTION

Xeric environments are areas where precipitation is restricted to a small part of the year. This includes arid and extremely arid habitats, characterized by a total annual precipitation of under 250 mm, but also ecosystems where, despite a total annual precipitation exceeding hundreds of mm, the long dry seasons causes the ground to dry out completely. Affecting the inhabiting biota is not only the extreme seasonal change in water availability, but also the fact that there is a massive inter-annual variation in the timing, occurrence and volume of rainfall regime (Shine and Brown 2008). Because of the large global extension of xeric lands and the impact of water scarcity on organism survival, such ecosystems are bound to promote well adapted life-forms.

Amphibians are particularly sensitive to environmental water availability due to their particularities in physiology, mainly related to permeable tegument and ectothermy (Feder and Burggren 1992), to their water-dependent reproduction (Duellman and Trueb 1994), and their reduced mobility compared to other vertebrates (Smith and Green 2005). However, amongst anurans, some species have adapted to xeric environments, either extremely arid or drastically seasonal and unpredictable in precipitation distribution, by adopting an obligate fossorial life-style. The present study aims at advancing the understanding of the complexity of life-history adaptations and behavioural strategies that allow fossorial anurans to survive in such demanding environments.

The thesis is structured into three general chapters and four specific ones. The general introduction gives an overview of the challenges imposed by xeric environments on their biota and on the amphibians in particular, and a synopsis of the accumulated knowledge regarding the adaptations of the amphibians that inhabit such places, with a focus on fossorial species from diverse geographic regions and regardless of phylogenetic origin. Each of the nine thematic subchapters deals with independent, but interrelated research questions and is structured as a stand-alone manuscripts. The subchapters are further grouped into four main chapters, each focused on one particular stage in the life-cycle of the study organism (larvae, juveniles, adults, and finally reproduction and egg-laying). The final general chapter summarizes and interconnects the main findings of the individual studies, and provides conclusions regarding the trade-offs imposed by life in xeric environments.

PRESENTATION OF THE STUDY SITE AND STUDY SPECIES

To test the research predictions, a seasonally dry tropical forest located in the Pacific Ecuador, Arenillas Ecological Reserve (03°34’ S, 80°08’ E, 30 m above sea level), was chosen as the study site. The region is characterized by severe climatic seasonality, consisting of a dry season that lasts from May to December, during which at least four months have less than 10 mm monthly precipitation, and a rainy season that lasts from January to April. Total annual amount of precipitation is on average 667 mm, but with large fluctuations (min. 65–max. 2647 mm/ year), especially since the area is under the influence of the El Niño Southern Oscillation. On the other hand, the temperature is more stable throughout the year, with an average of 25 °C.

The focus species for this project is the Pacific horned frog *Ceratophrys stolzmanni* (fig. 1), the only fossorial anuran present at the study site. This species belongs to the South American family Ceratophryidae, an anuran taxa comprised of three genera – *Ceratophrys*, *Chacophrys* and *Lepidobatrachus.* The clade is considered to have originated and diversified in a seasonally dry tropical environment (Faivovich et al. 2014).

Figure 1. (a) Illustration from the paper originally describing the species *Ceratophrys stolzmanni* - Steindachner (1882); (b) Adult Pacific horned frog from Arenillas Ecological Reserve, Ecuador.

All representatives of this family are fossorial, characterized by exceptionally wide and robust skulls (Fabrezi 2006), have macrophagous and facultatively carnivorous tadpoles (Fabrezi and Quinzio 2008) and short larval duration (Duellman and Lizana 1994; Quinzio et al. 2006).

CHAPTER 1

PLASTICITY IN LARVAL STAGES

1.1 DENSITY EFFECTS ON TADPOLE BEHAVIOUR, SURVIVAL AND POST-METAMORPHIC PERFORMANCE

Introduction

One of the most important mechanisms that determines population regulation is represented by the response to high density (Hixon et al. 2002). Amphibian larvae from ephemeral ponds are frequently subject to rapid increases in density, correlated with space restriction during pond drying. We investigated the effect of variation in larval density in a facultative cannibalistic tadpole, where density increase can be perceived as a cue both for intraspecific competition and possible predator pressure. We hypothesized that density in larval stage would generate changes in life-history (timing and size at metamorphosis), tadpole behaviour, as well as froglet morphology and behavioural performance. Our main predictions were that tadpoles raised at higher densities would: (1) experience higher mortality rates; (2) be more prone to cannibalism; (3) be less active, avoiding then attacks from siblings and (4) would metamorphose later or at a smaller size. We also tested if the shape or performance after metamorphosis is affected by the density treatment, beyond the outright effects of size.

Materials and methods

Larvae of *C. stolzmanni* were raised in an experimental setting at four density treatments: 1, 2, 4, and 8 tadpoles/ tank, and the effect of density was measured at three stages of their development. The tadpole mobility and rate of growth were measured mid-way through larval period (at the age of 8 days). Survival, age and size at metamorphosis were determined at the emergence of at least one forelimb. After the complete absorption of the tail, marking the finalization of the transition to terrestrial habitat, we checked for changes in shape of froglets and tested for differences in performance, reflected by gape size, jumping distance and endurance.

Main results and discussion

Tadpoles reacted to the density treatment early in their development, with larvae raised at higher densities growing slower (fig. 2a). The detrimental effect was maintained until metamorphosis, the crowded larvae suffering higher mortalities, undergoing metamorphosing later (fig. 2b) and experiencing slower growth rates (fig. 2c).

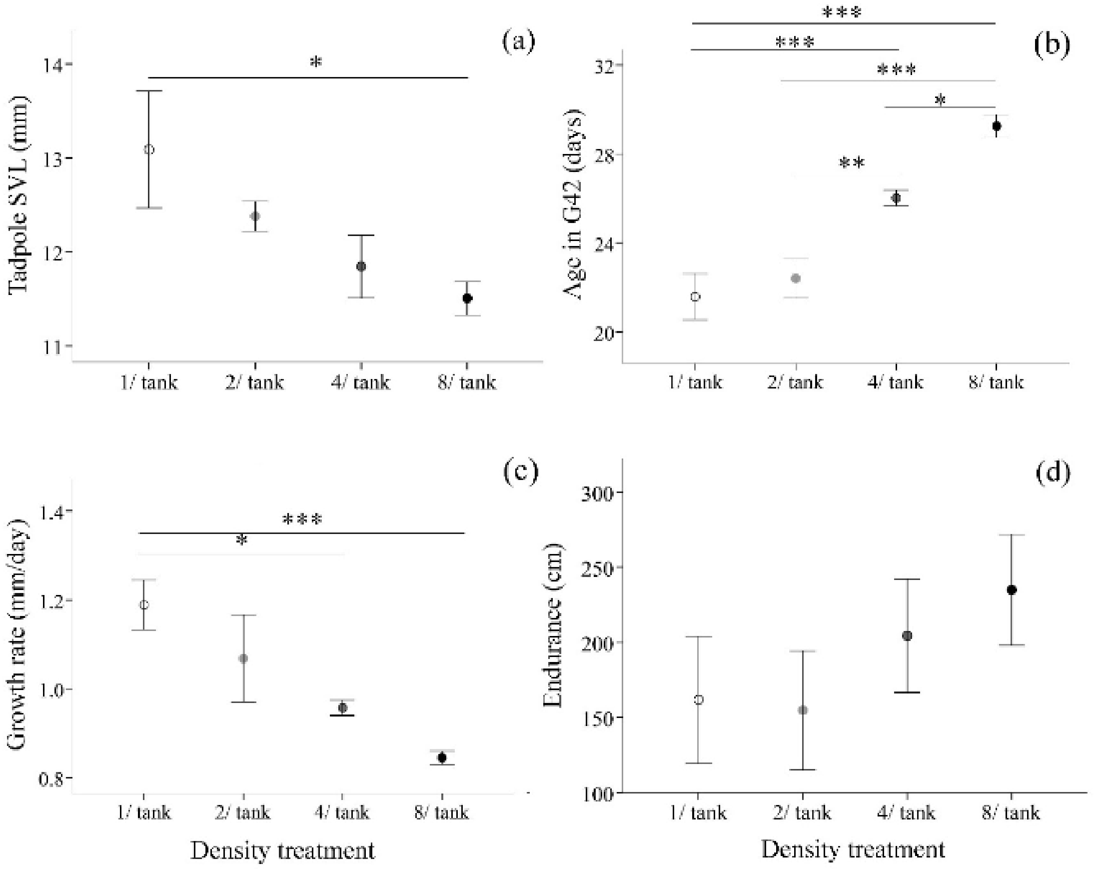

Figure 2. Performance in Pacific horned frogs (*Ceratophrys stolzmanni*) raised as tadpoles at different densities: (a) snout-vent length (SVL) at the age of 8 days; (b) age at metamorphosis; (c) average daily growth rates until metamorphosis; (d) post-metamorphic (G46 stage) endurance. Dots represent means, and whiskers SE. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. White dots - 1/ tank ($n = 12$), light grey - 2/ tank ($n = 12$), dark grey - 4/ tank ($n = 12$), black dots - 8/ tank ($n = 11$).

However, after the completion of metamorphosis, the individuals raised at high densities were able to travel longer distances before extenuation (fig. 2d), indicating that the conditions experienced during the larval development prepared them for better dispersion in terrestrial environments. Other investigated locomotor parameters were maintained similar regardless of density, such as swimming activity in tadpoles and jumping ability in froglets. Contrary to our prediction, the tadpoles of the Pacific horned frogs were not cannibalistic regardless of raising density, and their shape was not affected by experimental treatment.

1.2 POND DRYING CUES AND THEIR EFFECTS ON GROWTH AND METAMORPHOSIS

Introduction

The drying of breeding habitat before tadpole metamorphosis is frequently a source of mortality in pond-breeding amphibians, so rapid development is favoured in arid habitats such as deserts, where pond duration is short. However, because of inherent physiological constraints, an accelerated development can result in mortality before metamorphosis and is correlated with a small body size at metamorphosis, which can have a large impact on individual fitness afterwards (Morey and Reznick 2000; Altwegg and Reyer 2003). As a result, in more erratic xeric environments, where pond hydroperiod is highly unpredictable, phenotypic plasticity in larval duration is seen as particularly adaptive (Newman 1992; Padilla and Adolph 1996). By manipulating two cues of pond drying, we aimed at determining if: (1) tadpoles express phenotypic plasticity in both growth and developmental times until metamorphosis; (2) cues from a decreasing water level treatment and those from a low water depth treatment have similar effects; (3) faster development is correlated with smaller size at metamorphosis, and (4) mortality rates vary among the different treatments. Also, because tadpoles are particularly vulnerable between the start of metamorphosis, i.e. forelimbs emergence, and the stage when they can actually survive out of the water (Wassersug and Sperry 1977), we were interested to determine if they were able to accelerate the exit to the terrestrial habitat in response to perceived desiccation risk, an effect rarely monitored in experiments (Walsh 2010). We hypothesize that tadpoles should be able to adequately respond to desiccation risk and that both low water depth and decreasing water level induce a faster development and metamorphosis, at the cost of a smaller size at metamorphosis and higher mortality rates.

Materials and methods

In laboratory conditions, tadpoles were raised in one of three experimental treatments: constant high water level (2000 ml), constant low (500 ml) and decreasing water level (starting 2200 ml and decreasing daily until 250 ml). Their development was followed until two developmental stages: G42 (Gosner 1960) i.e. emergence of at least one forelimb, marking the beginning of metamorphosis, and G45 i.e. tail reduced to a stub, marking the moment when the larvae can survive in terrestrial habitat, avoiding mortality caused by the drying of the pond. At these stages, the parameters measured were age and body size, along with survival.

Main results and discussion

The tadpoles were able to time their metamorphosis in response to pond water level, reacting to both decreasing and low water levels.

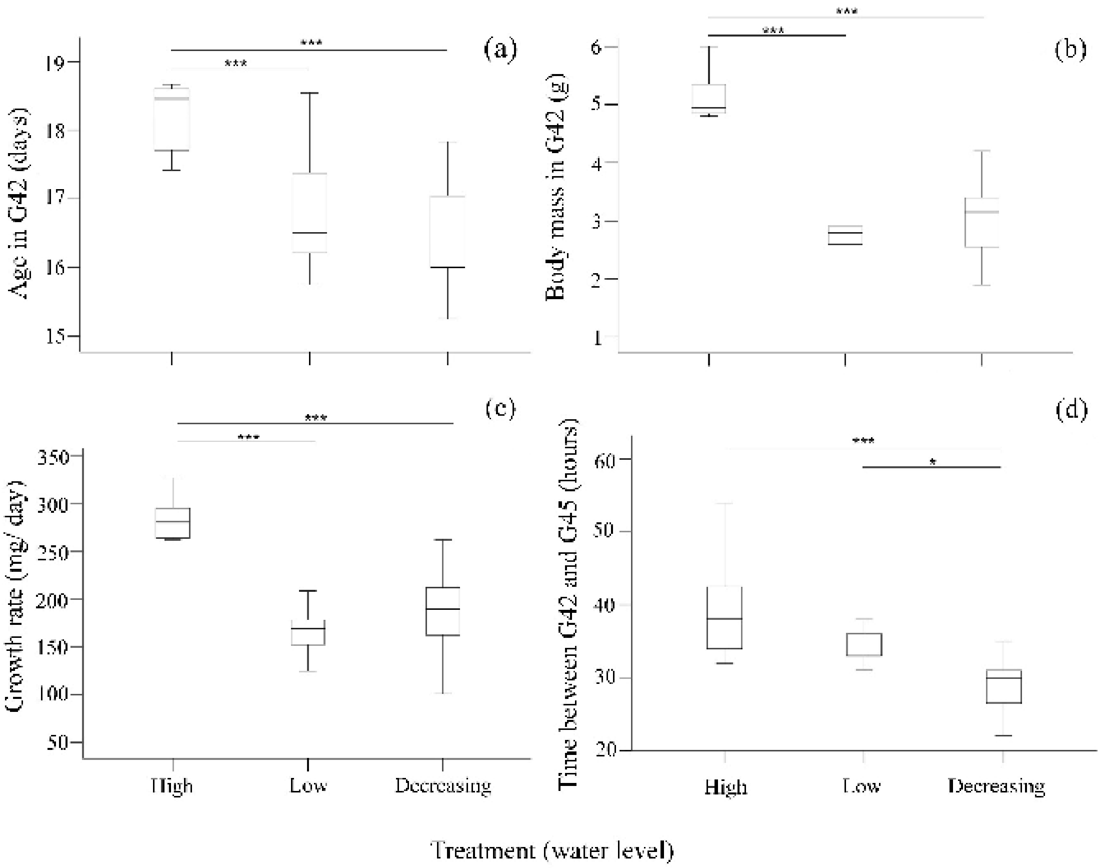

Figure 3. Life-history traits of Pacific horned frog tadpoles raised in different water level treatments: constant high water level, constant low water level, decreasing water level. (a) age of tadpoles in G42 developmental stage;

(b) tadpole body mass in G42; (c) estimated tadpole growth rate until G42; (d) interval of time necessary for tadpoles to pass from G42 to G45 developmental stage (i.e. the last stages of metamorphosis). Box plots show the median (dark midline) and spread of values, with boxes encompassing percentiles 25–75 and whiskers denoting minimum - maximum value not higher than 1.5 times the interquartile range. **p* < 0.05, ****p* < 0.001.

The individuals exposed to desiccation risk developed more rapidly (fig. 3a) and were able to leave the water sooner, but did so at a smaller size (fig. 3b). On the contrary, tadpoles encountering favourable conditions represented by the high water level, capitalized on the opportunities for additional growth, postponed their transition, and showed the highest rates of growth reported for any anuran species (fig. 3c). Additionally, the interval between the start of metamorphosis and the moment when larvae are able to leave the water, when tadpoles are most vulnerable because of limited mobility (Wassersug and Sperry 1977), was significantly shorter in the case of tadpoles experiencing a decrease in water level (fig. 3d).

CHAPTER 2

JUVENILE STAGE AND REACH OF MATURITY

2.1 ADULT - JUVENILE INTERACTIONS DETERMINE A TEMPORAL NICHE PARTITIONING BETWEEN LIFE-STAGES

Introduction

Divergence in ecological niche offers organisms the opportunity of exploiting differently the food and habitat resources and reduces competition both among species and among different age-classes of the same species (Lima et al. 1998; Eskew et al. 2009). Fossorial frogs limit their emergence from burrows to short intervals during or after rainfalls; as a result, at the study site, the number of opportunities for foraging is limited because of the short duration of the rainy season. Additionally, the species is anurophagous in terrestrial stages and its activity is limited to the level of the ground, suggesting the potential for competition and predation between juveniles and adults. In this context, we tested the ontogenetic divergence in ecological niche, through the adoption of contrasting diel patterns. We hypothesize that, in order to avoid competition with larger conspecifics, and also to reduce the risk of being cannibalized by adults, a temporal partitioning between adult and juvenile life-stages is present in the population. Additionally, we investigated the body condition of juveniles active during various intervals of the diel cycle, predicting that the individuals with lower body condition should be forced to extend their activity even under the most unfavourable climatic conditions.

Materials and methods

Field observations were carried out on a 2.5 km transect, censusing active individuals both during the day and during the night. The study took place from the start of the activity season, when adults come out of aestivation, up to a week after the onset of mass metamorphosis. To test the cannibalistic hypothesis, the field study was complemented by a laboratory experiment (fig. 4a), designed to follow the juveniles' behavioural changes brought about by the visually perceived presence of adults. We monitored the mobility of freshly metamorphosed individuals that were visually exposed to an adult in their vicinity, and compared their behaviour with juveniles put in an identical setting, only without the adult.

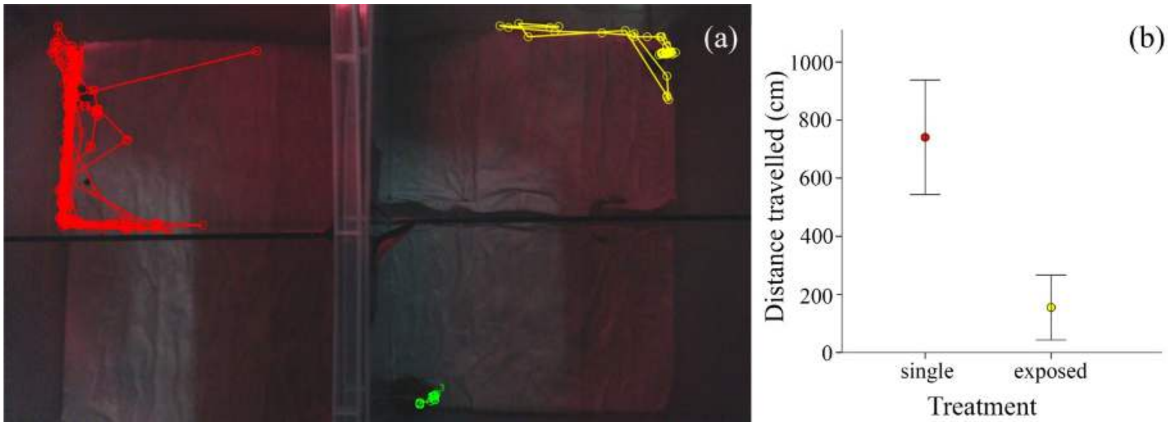

Figure 4. Influence of perceived adult presence on juvenile activity: (a) Experimental design: left enclosure - single treatment, with the juvenile in the upper part (red track) and an empty arena in the lower part. Right enclosure - exposed treatment, with a juvenile in the upper arena (yellow track) and an adult in the lower arena (green track). Arenas are separated by transparent glass, and there is no visibility between the enclosures. The coloured paths indicate the distance travelled by individuals over the course of one hour. (b) Distance travelled (mean ± S.E.) in experimental enclosures during one hour by single juveniles - that could not visually perceive any adult in their vicinity, and exposed juveniles - that could see an adult in their close vicinity.

Main results and discussion

Immediately after leaving water, the froglets preferentially adopted a diurnal life style, which contrasts with the strictly nocturnal activity of older horned frogs. Especially smaller individuals, with a lower body condition, were active during the day, in spite of meteorological conditions generally considered detrimental, i.e. higher temperatures and lower relative humidity. On the other hand, in the laboratory experiment, froglets exposed to the presence of an adult showed a marked tendency to reduce movement (fig. 4b); the lack of movement can be interpreted as an antipredator response, supporting the hypothesis that the divergence in temporal niche use between age-classes is determined by intra-specific interactions, and serves as a modality to reduce predation pressure and competition for resources.

2.2 CARRY-OVER EFFECTS ACROSS AQUATIC AND TERRESTRIAL LIFE-STAGES: SIZE AT METAMORPHOSIS AFFECTS GROWTH AND SURVIVAL

Introduction

Species with complex life cycles, such as biphasic amphibians, are able to exploit different ecological niches and optimize their life-history in discrete developmental stages (Wilbur 1980; Laudet 2011). Size at metamorphosis is a key life-history trait, which reflects the complex interaction between the costs and benefits of life in the aquatic and terrestrial environments and has a profound effect on individual fitness (Wilbur and Collins 1973; Altwegg and Reyer 2003; Rudolf and Rödel 2007). Following the observation that conditions experienced during the larval stage determine a broad range of sizes at metamorphosis, the effects of this trait on performance during the following terrestrial stage were investigated in a laboratory experiment. We evaluated the impact of extreme size phenotypes on the short and medium-term fitness, during the first activity season of juveniles, before the start of the dry season, during which horned frogs aestivate in underground burrows. We tested if smaller froglets can mediate the detrimental survival effects induced by metamorphosing at a reduced size through (*i*) allometric changes in morphology or performance (wider heads, longer limbs, and better jumping skills) and (*ii*) higher growth rates.

Materials and methods

We selected the smallest and largest metamorphosing individuals encountered in the natural habitat, and raised them in laboratory for two months, providing environmental conditions that would favour growth, i.e. *ad-libitum* food and high humidity. The measured fitness-related parameters chosen for the study were mobility and gape width at the completion of metamorphosis, survival until the end of the experiment, equalling an activity season, and growth rates.

Main results and discussion

We found that size at metamorphosis deeply affects the fitness of horned frogs in their juvenile life-stage. Individuals that were larger at metamorphosis were more mobile and had wider heads, suggesting better performance in escaping predators and acquiring food. They also showed better survival rates for the duration of the first activity season (fig. 5a). However, smaller individuals were able to intensify their growth rates (fig. 5b) and reduced the initial size gap before their first aestivation.

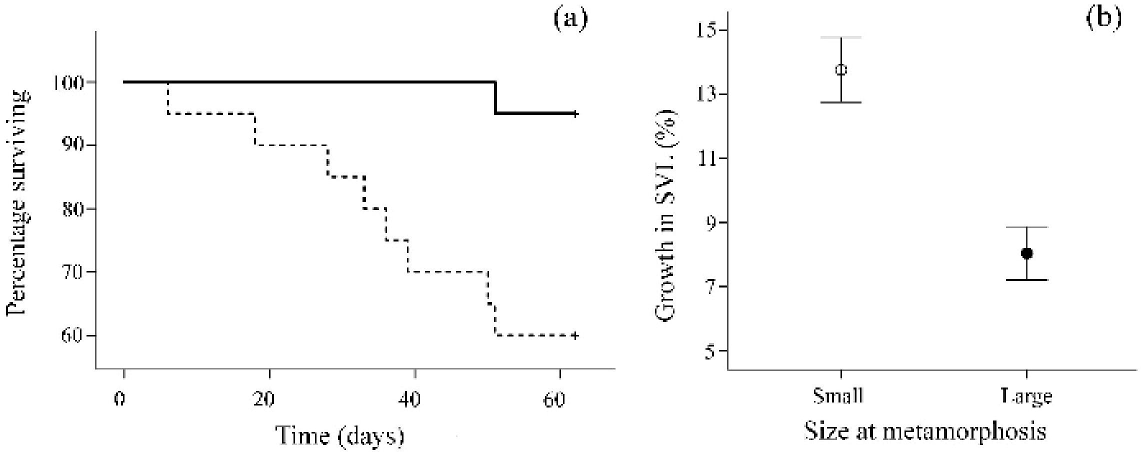

Figure 5. Survival and growth over the experimental period (62 days) in juveniles of *Ceratophrys stolzmanni*, depending on their size at metamorphosis: (a) Kaplan-Meier survival curves: dotted line - small metamorphosing juveniles; full line - large metamorphosing juveniles; (b) growth (mean ± SE increase in SVL).

Introduction

Effective conservation requires understanding species' natural history and identifying factors that influence extinction risks and the ability to recover after perturbations (Selwood et al. 2015). Life-history traits, like longevity, age at first reproduction, and body size are amongst the most important parameters for the study of populations and a prerequisite for designing effective conservation strategies (Biek et al. 2002). In this context, we investigated for the first time the growth and maturation patterns, as well as the age structure and longevity in the studied population.

Materials and methods

The study is based on information collected from 153 adult and 92 juvenile individuals encountered during the rainy season of 2015 at the study site. The skeletochronological method was used to assess the age of adults. Post metamorphic growth pattern was deduced from von Bertalanffy's growth model and bone growth parameters.

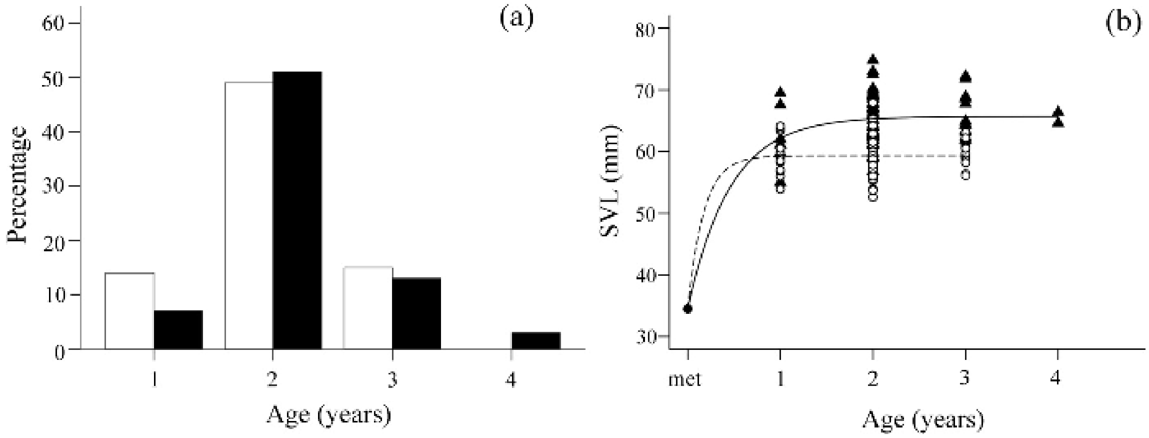

Figure 6. (a) Age structure of *Ceratophrys stolzmanni* in Arenillas Ecological Reserve, Ecuador, determined by skeletochronology. Open bars - males, full bars – females; **(**b) Growth described by Von Bertalanffy´s model in *C. stolzmanni* males (open circles, dotted line) and females (full triangles, smooth line). met = metamorphosis, full circle = average size at metamorphosis.

Main results and discussion

Longevity was low, with a population mean age of two years in both sexes, and only 2% of tested individuals surviving to four years (fig. 6a). The intense growth rates experienced during the short larval period were maintained during the first activity season (fig. 6b). In spite of a long aestivation, both males and females reached sexual maturity during the first year of life.

CHAPTER 3

BEHAVIOURAL ADAPTATIONS TO DRY ENVIRONMENTS IN TERRESTRIAL STAGE

3.1 DRYNESS AFFECTS BURROWING DEPTH

Introduction

Fossorial life is considered to be a behavioural strategy that offers protection from unfavourable environmental conditions such as extreme temperatures and dehydration (Bentley 1966; Kinlaw 1999). Owing to their permeable skin, amphibians can exchange water from surrounding soil (Ruibal et al. 1969; Booth 2006). As a result, burrowing anurans should choose the depth of their burrows depending on the moisture of the substrate. We hypothesized that frogs burrowing in moist soil would conserve energy and permanent in shallow layers, but, when exposed to dry conditions, they would dig deeper into the substrate to seek moisture and rehydrate.

Materials and methods

In a laboratory setting, we used experimental enclosures that provided the frogs with a large range of depth for burrowing (up to 130 cm), but allowed precise observation of the location of the individual at all times (fig. 7a). Individuals were assigned to either the dry (10%) or the humid (50%) substrate treatment. Their behaviour was monitored for two consecutive nights.

Main results and discussion

Pacific horned frogs regulated their burrow depths depending on substrate humidity. When humid substrate was available, the frogs remained in close vicinity to the surface, however, when the substrate was dry, their response was to dig deeper in search of more favourable osmotic conditions (fig. 7b). They were able to excavate rapidly, reaching more than one-meter depth in a few hours.

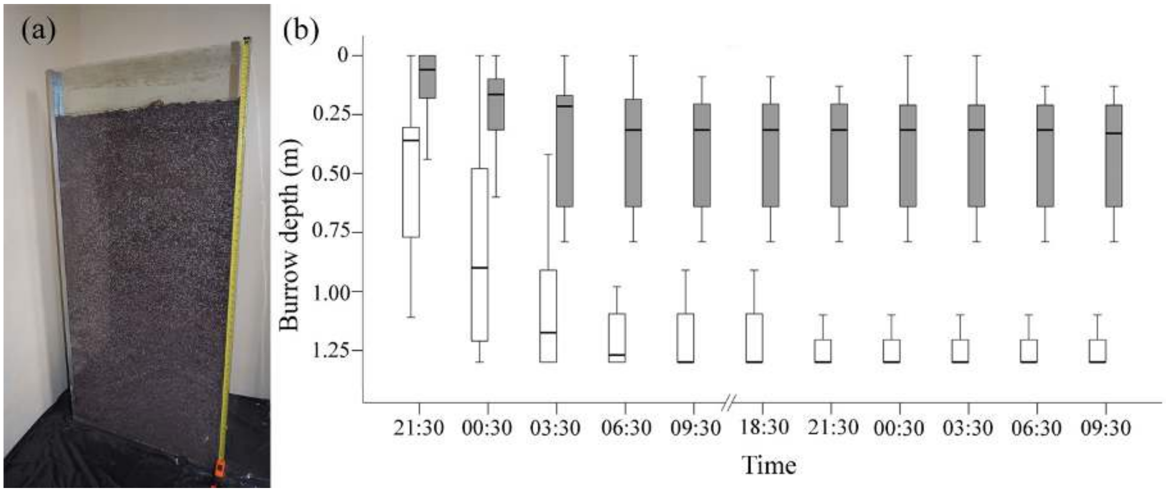

Figure 7. (a) Experimental design and (b) burrowing depth in *Ceratophrys stolzmanni* from wet (dark boxes) and dry (light boxes) treatment (*n* = 12/treatment) over the experiment duration (39 hours), that started at 18:30 when individuals were introduced in experimental enclosures. Interval between 9:30 and 18:30 (during daylight) was not checked. Box plots show the median (dark midline), percentiles 25–75 and minimum–maximum value not higher than 1.5 IQR.

3.2 FOSSORIAL AMPHIBIANS IN XERIC ENVIRONMENTS - SPATIALLY-RESTRICTED, PRECIPITATION-DEPENDENT ACTIVITY

Introduction

Activity levels in most species are determined by climate, individuals showing large variations in foraging behaviour depending on both diel and seasonal changes in environmental conditions (Ni et al. 2015; Vlašánek et al. 2018). In highly seasonal environments, where activity is possible only for a short interval of time because of physiological restrictions, the dependence between climate and activity patterns is exacerbated (Green et al. 2016), and individuals are active for a large proportion of the time when conditions are physiologically tolerable (Timm et al. 2014). Fossorial frogs spend most of their lives underground, where they are protected from predators and sheltered from adverse climatic conditions. Emergence from burrows and movement in these species is considered mainly related to foraging and reproductive activities (Huey and Pianka 1981; Zug et al. 2001; Wells 2010). In this context, we conducted a capture-mark-recapture study, aiming to: (1) identify the environmental factors driving frog activity; (2) estimate mobility, body condition and growth-rates for individual adult frogs; and (3) estimate population abundance and survival.

Materials and methods

Using a capture-mark-recapture design based on photographic recognition, we monitored the adult frogs from a 2.5 km transect at the study site, for two months, during the rainy season. Each encountered individual was measured, photographed and the GPS coordinates of its location were taken. We collected several environmental factors, such as temperature, relative humidity, precipitation occurrence and quantity, as well as diel interval.

Main results and discussion

Adult horned frogs were strictly nocturnal, and the main determinant of emergence from burrows was precipitation, even during the rainy season the majority of horned frogs being active only during or shortly after rainfall. The two sexes had similar patterns of activity, both in terms of time spent outside their burrows, and of total area used. Individuals showed relatively high site-fidelity and low mobility, with small total area of activity and high apparent survival rates. The body condition of individuals improved as the rainy season progressed (fig. 8), suggesting an increase in foraging opportunities brought about by water availability. Neither total distance travelled nor the activity areas were influenced by the frog size or body condition. Estimated abundance of frogs at the location is 1461 adults/ha. From the estimated number of individuals present on the transect, 32.5% were captured and marked at some point during the two months of monitoring. Recaptures represented 25% of the sightings, occurring between 1 and 46 days after the initial sighting.

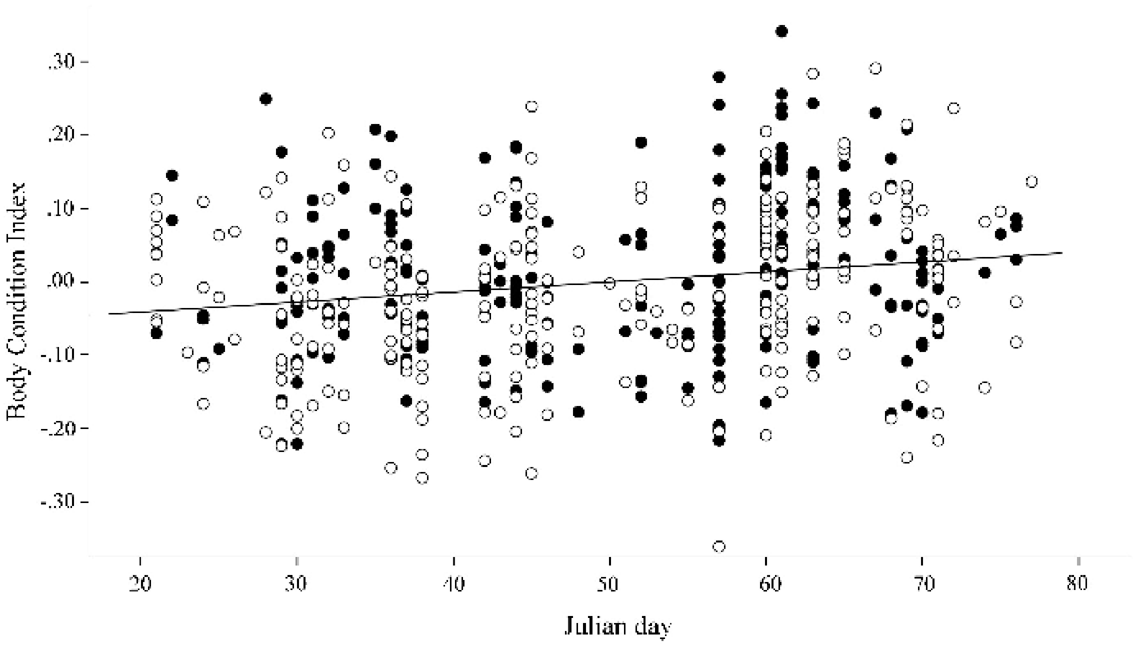

Figure 8. Relationship between day of the year and individual body condition in the *Ceratophrys stolzmanni* population throughout the study period. Males - black dots, females - white dots.

3.3 WHAT DOES A PACMAN EAT? MACROPHAGY AND NECROPHAGY IN A GENERALIST PREDATOR (*CERATOPHRYS STOLZMANNI*)

Introduction

Information regarding the feeding ecology and foraging strategy of a species is essential in understanding its importance in the ecosystem. The frogs belonging to the family Ceratophryidae are characterized by morphological traits such as huge, robust skull proportionally to their body size (thus the name of Pacman frogs given as vernacular to *Ceratophrys* species), a reduced skull length associated with an increase in skull width (Fabrezi 2006) and fangs (Fabrezi and Emerson 2003), which allow them to ingest large prey.

Materials and methods

The study is based on non-invasive methods, using direct observation of foraging behaviour in the natural habitat and gastro-intestinal contents analysis of frogs encountered dead.

Main results and discussion

Like its larger congeners, the horned frogs consumed a diversity of prey, which included invertebrates of various size and type, with a predominance of gastropods, non-formicid hymenopterans and centipedes, as well as amphibians and reptiles. Both freshly metamorphosed juveniles and adults attacked anurans, including individuals of the same species (fig. 9).

Figure 9. Anurophagy in *Ceratophrys stolzmanni*. (a, b) Cannibalism in juveniles; (c) adult *C. stolzmanni* preying upon a *Leptodactylus labrosus*; (d) adult *C. stolzmanni* scavenging on a *Trachycephaus jordanni* carcass.

Additionally, they engaged in necrophagy, an exceptional behaviour for terrestrial anurans (fig. 9d). We found no relationship between the size of the frog and the size of its prey, even large individuals consuming small items such as mites.

CHAPTER 4

REPRODUCTIVE STRATEGIES

4.1 RANDOM SIZE-ASSORTATIVE MATING DESPITE SIZE-DEPENDENT FECUNDITY IN A NEOTROPICAL AMPHIBIAN WITH EXPLOSIVE REPRODUCTION

Introduction

In species where size or age are correlated with higher fitness or fecundity, the choice of sexual partners based on these parameters can carry important benefits (Kokko et al. 2003). However, in explosive-breeding systems, where mating opportunities are available for very short intervals of time, the active choice of partners involves the risk of losing an entire reproductive event. In xeric environments, where permanence of water bodies available for egg-laying is unpredictable, there is a strong selective pressure for highly synchronized, short lasting, breeding events. At the study site, each year, the Pacific horned frogs breed explosively only during one night following the first heavy storm of the rainy season. Limiting even more their reproductive opportunities is the fact that individuals have a low life expectancy. As a result, we predicted the existence of a female biased sexual dimorphism, and a large female investment in the production of egg-clutches. In terms of mate choice, we investigated the effect of size, age or body condition on the reproductive success of individuals.

Materials and methods

Field surveys at the same breeding pond were carried out over two consecutive years. The existence of assortative mating, i.e. a preference of larger (or older) males to pair with larger females, was tested by measuring and aging individuals that were found in amplexus. Female investment was estimated by comparing the female size to the number of eggs it laid and the mass loss after reproduction. To test if size or body condition influences male reproductive success, after all females from the breeding pond were in amplexus, all solitary males were measured and their morphological parameters were then compared to the ones found in pairs.

Main results and discussion

We found no evidence of a size-assortative mating system, since larger males were not able to secure larger females (fig. 10a), in spite of the fact that the reproductive output of females depended on their size. Additionally, the success of males in attracting females and achieving amplexus was not influenced by their size, body condition or age (fig. 10b). Together, these results indicate that in systems where breeding opportunities are rare and short-lasting, the random mating pattern is favoured, despite theoretical advantages of active mate choice.

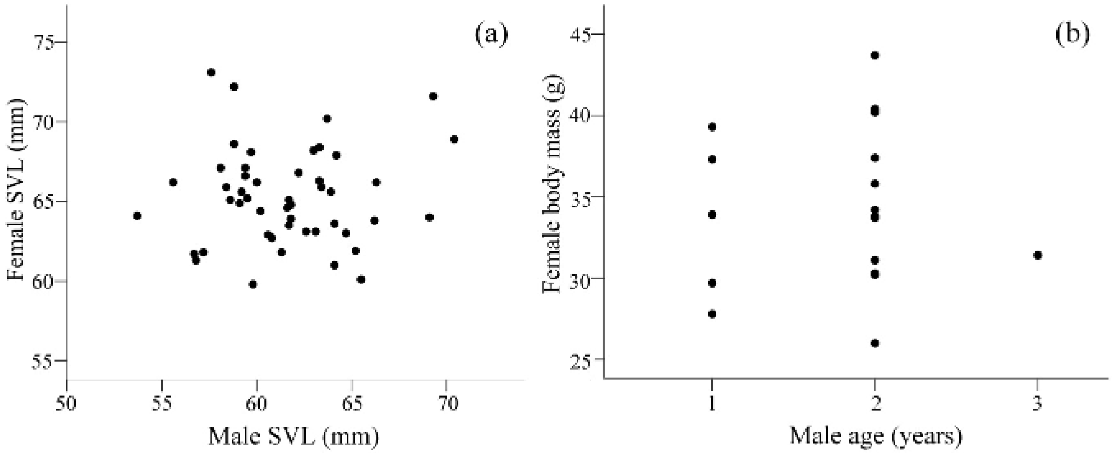

Figure 10. (a) Non-assortative mating (snout-vent length of partners found in amplexus, n = 49) and (b) female body mass in relation to the age of their partners (n = 19) in *Ceratophrys stolzmanni.*

CONCLUSIONS

We found that several aspects of the life of horned frogs are profoundly affected by and finely tuned to the seasonal patterns of rainfall and to the changes in water availability in their habitat. Limitations in terms of rainfall quantity affected both terrestrial and aquatic life-stages, adults drastically reducing their activity and tadpoles modifying their developmental pathways, adopting a rapid metamorphosis that was linked to fitness reduction in later stages, and juveniles maturing early, with a correlated reduction in longevity. However, several strategies allow the horned frogs to capitalize on growing opportunities whenever such are available, by diversifying the modalities of resources exploitation, synchronizing their reproduction to a short interval to give tadpoles the longest larval interval possible, and reducing their energetic expenditures through low mobility and flexible depth of burrows. Our results indicate that the harsh conditions correlated with xeric environments trigger consistent adaptations in anurans that adopt the burrowing life history, confirming observations made in other fossorial amphibians living in habitats that suffer from severe water limitations.

Our study enhances the understanding of this poorly-known species and of the potential risks to its long-term survival. Due to the strong influence of rainfall regime on all life-stages, the predicted changes in precipitation patterns are likely to lead to further restriction of the activity of the frogs and to reductions of recruitment or juvenile fitness. Additionally, the short reproductive lifespan of the species makes the persistence of the population vulnerable to persistent droughts of 2–3 consecutive years because of low recruitment. The population parameters that we provide in this thesis serve as base-line information for the present welfare of the population, and can be a starting point for the detection of potential declines. This knowledge should be included in future management plans for the effective conservation of the species.

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Invited talks:

"*Estudios a largo plazo de las poblaciones de anfibios y reptiles en Rumania*". On 27 October 2014 at National University of Misiones, Posadas, Argentina and on 3 November 2014 at National University of North-East, Corrientes, Argentina.

"*Behavioral strategies in amphibians living in xeric environments: an analysis of movement and resource use across life stages*". On 5 October 2016 at Universidad Técnica Particular de Loja, Loja, Ecuador.