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"HISTÓRIA NATURAL DE *RHINELLA JIMI* (ANURA; BUFONIDAE): UMA ESPÉCIE INVASORA EM FERNANDO DE NORONHA"

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Orientador: Dr. Luís Felipe de Toledo Ramos Pereira

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Resumo. A introdução de espécies exóticas de anfíbios é um fator que tem se mostrado preocupante por contribuir para o desequilíbrio de comunidades animais nativas. Estas espécies além de se alimentarem de espécies nativas, podem se multiplicar rapidamente devido à ausência de predadores e competidores, podem ainda transmitir doenças às espécies nativas e causar problemas devido à hibridização com espécies aparentadas. *Rhinella jimi* é um Bufonidae do grupo de *R. marina* que foi introduzido no arquipélago de Fernando de Noronha há, no máximo, 120 anos. Esta população apresenta alta prevalência de deformidades, sendo que quase 50 % dos indivíduos adultos apresentam alguma anomalia externa. Cerca de 20 % destes possuem problemas nos olhos, sendo alguns completamente cegos. O objetivo principal deste trabalho foi estudar aspectos da história natural e biologia reprodutiva da população de *Rhinella jimi* de Fernando de Noronha, considerando a alta prevalência de deformidades nesta população.No primeiro capítulo foi feita a descrição do estágio larval de *Rhinella jimi*, necessária para a avaliação de deformidades, realizada no segundo capítulo. O terceiro capítulo trata-se de um estudo da dieta desta população, baseado em dados de conteúdo estomacal complementados por análises de composição isotópica. No quarto capítulo foi feita uma investigação da forma com que os indivíduos cegos localizam e capturam as presas, bem como das possíveis consequências que os problemas de visão podem trazer para as condições físicas dos animais. No quinto capítulo foi realizado um estudo de reprodução, com descrição das vocalizações e dos comportamentos observados. Os resultados mostraram uma alta prevalência de deformidades entre os girinos, com mais da metade dos indivíduos deformados. As deformidades no estágio larval ocorrem principalmente no disco oral e estão ligadas a um menor tempo de forrageio e uma menor eficiência na aquisição de alimento. Apesar da ausência de presas no estômago de indivíduos cegos, o estudo de dieta dos adultos mostrou que estes têm a dieta mais parecida com a dos indivíduos caolhos (com apenas um olho funcional). Indivíduos caolhos e indivíduos sem deformidades (normais) apresentaram formigas como as principais presas, mas Diplopoda foi um grupo importante na dieta de caolhos, enquanto para os indivíduos normais Coleoptera se mostrou mais abundante. O comportamento alimentar diferiu entre indivíduos cegos (que caçam por espreita com base em estímulos táteis) e normais (que caçam ativamente baseados em estímulos visuais). Os problemas de visão também influenciam a massa corporal, com indivíduos cegos tendo menor massa. No estudo de reprodução observamos a ocorrência de dimorfismo sexual de tamanho (fêmeas maiores que os machos) e uma tendência ao maior esforço reprodutivo e número de ovos em indivíduos normais. Foram observadas duas estratégias reprodutivas adotadas pelos machos (tanto normais como cegos), a de vocalização e a de procura ativa por fêmeas. No geral, tanto girinos como adultos são prejudicados pelas deformações, e estas deficiências poderiam implicar em eliminação de indivíduos menos adaptados no âmbito da sua distribuição geográfica natural. No entanto, a população invasora e ilhada está conseguindo sobrevier e reproduzir com sucesso, mantendo altos níveis de deformidade.

Abstract. The introduction of alien amphibian species has shown concern as it may contribute to the imbalance of native animal communities, causing environmental damage. These species feed on native species, breed rapidly due to the absence of predators and competitors, may also transmit diseases to native species and cause problems associated to hybridization with related species. *Rhinella jimi* is a Bufonidae of *R. marina* species group that was introduced in the archipelago of Fernando de Noronha near 120 years ago. This population presents a high prevalence of deformities, as almost 50% of adults have some external anomaly. About 20% of them present eye problems, some being completely blind. The main objective of this research was to study the natural history and reproductive biology of the population of *Rhinella jimi* from Fernando de Noronha, considering the high incidence of deformities among the individuals of this population. In the first chapter we described the tadpoles of *R. jimi*, necessary for the assessment of deformities evaluated in the second chapter. The third chapter was a study of the diet of this population, based on data from stomach contents complemented by isotopic composition analysis. In the fourth chapter we investigated the way that blind individuals locate and capture prey, as well as the possible consequences that vision problems may have on the physical conditions of animals. The fifth chapter is a study of reproduction, with descriptions of vocalizations and behaviors. The results showed a high prevalence of deformities among tadpoles, with more than half of them being deformed. Deformities in larval stage occur mainly in the oral disc and are connected to a shorter time foraging and a lower efficiency in the acquisition of food. Despite the absence of prey in the stomach of blind subjects, the study showed that these toads have a diet similar to the half blind individuals (with only one functional eye). Half blind individuals and individuals with no deformity (normal) had ants as the main prey, but Diplopoda was an important item in the diet of half blinds, while for normal individuals Coleoptera was more abundant. The feeding behavior differed between normal and blind individuals, with blind ones using tactile stimuli and a sit-and-wait foraging strategy, and normal ones using visual stimuli and active search foraging strategy. Vision problems also affect the body mass of the toads, as blind individuals had lower body mass. We observed the occurrence of sexual dimorphism in size, with females larger than males, and a trend of higher egg production and reproductive effort in normal individuals. We observed two reproductive strategies adopted by males, both blind and normal individuals, the vocalization and the active search for females. Deformed individuals (tadpoles and adults) present disadvantages in comparison to normal ones. In all cases half blind individuals presented intermediate patterns between normal and blind ones. The extent of disadvantages observed could imply in individual elimination in the natural range of this species. However, in the island the conditions may support their existence up to successful reproduction.

Conteúdo

Introdução geral

O Arquipélago de Fernando de Noronha se situa a cerca de 400 km da costa nordeste do Brasil (03º50'S, 32º25'W) e pertence ao estado de Pernambuco. É formado por um complexo de 21 ilhas e ilhotas sendo que a maior delas (Ilha de Fernando de Noronha) tem 10 km de comprimento e 27 km^2 de área. O arquipélago tem origem vulcânica, tendo surgido há cerca de 12 milhões de anos, e está situado sobre o pico de uma montanha submersa cuja base está a cerca de 4.500 m de profundidade (Bonaldo, 2005). O seu clima é quente oceânico, com temperatura média de 28 ºC e duas estações bem definidas, uma chuvosa (inverno - de março a agosto) e uma seca (verão - de setembro a fevereiro) (Silva, 2007). Não existem nascentes no arquipélago e o abastecimento de água doce é feito através de açudes, sendo o maior deles o Açude do Xaréu (Silva, 2007). Existem ainda pequenos riachos, como o Mulungu, o Maceió e o Boldró, que secam durante o verão (Silva, 2007).

No arquipélago de Fernando de Noronha existem diversas espécies que foram introduzidas pelo homem, como o lagarto teiú (*Tupinambis merianae*), a lagartixa-de-parede (*Hemidactylus mabouia*), o mocó (*Kerodon rupestris*), invertebrados terrestres e duas espécies de anfíbios, a perereca-de-banheiro (*Scinax x-signatus*) e o sapo-cururu (*Rhinella jimi*) (Silva, 2007; L. F. Toledo, com. pessoal). A introdução de espécies exóticas de anfíbios é um fator que tem se mostrado preocupante por contribuir para o desequilíbrio de comunidades animais nativas, causando prejuízos ambientais (Ramos et al. 1994; Bebee, 1996; Crossland, 2000). Estas espécies além de se alimentarem de espécies nativas, podem se multiplicar mais rapidamente devido à ausência de predadores e competidores, e ainda podem transmitir doenças a espécies nativas e causar problemas devido à hibridização com espécies aparentadas (Boelter & Cechin, 2007). Os mais notáveis exemplos de introduções de anfíbios anuros são os casos da rã-touro norte americana (*Lithobates catesbeianus*) e do sapo-cururu (*Rhinella marina*), que estão entre as 100 piores espécies exóticas invasoras do planeta (Lowe et al., 2000).

Rhinella jimi (Stevaux, 2002) é um Bufonidae do grupo de *R. marina* que tem sua distribuição original no nordeste do Brasil (Frost, 2011), sendo sua localidade-tipo a cidade de Maracás, Bahia (Stevaux, 2002). A introdução de *R. jimi* é relativamente recente na ilha, tendo ocorrido a, no máximo, 120 anos (Olson, 1981). Segundo moradores locais, a espécie pode ter sido introduzida com objetivo de controle de insetos-praga, pelo padre Francisco Adelino de Brito Dantas, que morou na ilha principal nas décadas de 1870 e 1880 e possuía uma pequena lavoura. Atualmente a população de *R. jimi* encontra-se distribuída por toda a ilha principal. Recentemente, Toledo & Ribeiro (2009/2010) identificaram uma alta ocorrência de indivíduos com malformações nesta população, sendo que quase 50 % dos indivíduos adultos apresentam alguma anomalia externa. Cerca de 20 % destes, possuem problemas nos olhos, sendo alguns completamente cegos. Sabe-se que a captura de alimentos é dependente da visão no gênero *Rhinella* (Haddad et al., 1990), isto pode levar indivíduos com problemas de visão a terem baixo ganho de peso e problemas no crescimento, e, como o tamanho corporal pode influenciar de muitas maneiras na reprodução dos anuros, pode afetar o desempenho reprodutivo destes indivíduos.

A ampla distribuição, a grande variedade de formas e o alto grau de ocorrência de malformações em anuros têm recentemente chamado a atenção de cientistas do mundo todo (Meteyer, 2000; Blaustein & Jhonson, 2003). Dentre os principais tipos de anomalias observadas estão a falta ou excesso de membros e dedos, formação incompleta dos membros, olhos deformados e lesões na pele (Meteyer, 2000; Blaustein & Jhonson, 2003; Lanno, 2008). Várias causas são apontadas como responsáveis pelo aparecimento destas anomalias e há certo consenso de que se trata de malformações congênitas induzidas por fatores ambientais (Gardiner & Hoppe, 1999). O aumento na radiação UV vem sendo apontado como possível causa de deformidades nas pernas de anuros (Ankley et al. 2000; 2002). Outra causa possível é a exposição a doses sub-letais de pesticidas, como observado em larvas de *Scinax nasicus* expostas ao herbicida glifosato, que apresentaram anormalidades craniofaciais, bucais e nos olhos (Lajmanovich et al., 2003) e em larvas de *Pelophylax ridibundus* submetidas a tratamento com o inseticida malatiom (Sayim, 2008). Jhonson et al. (1999; 2001) relataram a ocorrência de anomalias em *Pseudacris regilla* devido à infecção pelo verme trematódeo *Ribeiroa ondatrae*. Em populações pequenas e/ou isoladas a proporção de cruzamentos entre indivíduos aparentados (endocruzamentos) é alta, o que potencializa o aparecimento de caracteres deletérios na população (Futuyma, 1992; Begon et al., 2007), portanto, nestas populações, a endogamia pode ser uma das causas do aparecimento de malformações.

O objetivo principal deste trabalho foi estudar aspectos da história natural e biologia reprodutiva da população de *Rhinella jimi* de Fernando de Noronha, considerando a alta ocorrência de deformidades entre os indivíduos desta população. Os objetivos específicos foram: i) Descrição das larvas de *Rhinella jimi*; ii) Verificação da ocorrência de malformação nas larvas de *R. jimi*; iii) Estudo do comportamento alimentar de indivíduos cegos, com apenas um olho funcional e normais de *R .jimi*, verificando possíveis diferenças; iv) Identificação das estratégias reprodutivas adotadas pela população local de *R. jimi*, comparando as estratégias adotadas por indivíduos saudáveis àquelas adotadas pelos que têm problema de visão; v) Comparação do esforço reprodutivo entre fêmeas cegas e saudáveis; vi) Comparação do sucesso reprodutivo, do deslocamento e do tamanho corporal de indivíduos cegos, com apenas um olho funcional e saudáveis da população.

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CAPÍTULO 1 - Tadpole of *Rhinella jimi* (Anura: Bufonidae) with Comments on the Tadpoles of Species of the *Rhinella marina* Group

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Tadpole of *Rhinella jimi* (Anura: Bufonidae) with Comments on the Tadpoles of Species of the *Rhinella marina* Group

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Abstract. Here we describe the tadpoles of *Rhinella jimi. R. jimi* tadpoles are benthic and exotrophic, and display aggregative behavior. These tadpoles can be distinguished from other members of the *R. marina* group by the combination of the following characters: spiracle with external tube opening on midbody, snout sloped in lateral view, eyes and nostrils proportionally larger than in *R. schneideri*. Finally, we reviewed the information available on the other described tadpoles of the *R. marina* species group and compare them with the tadpole of *R. jimi*.

The genus *Rhinella* Fitzinger is currently composed of 77 species distributed in North, Central, and South America (Frost, 2009). The *R. marina* species group (*sensu* Martin, 1972) is composed by ten species: *R. achavali*, *R. arenarum*, *R. cerradensis*, *R. icterica*, *R. jimi*, *R. marina*, *R. poeppigii*, *R. rubescens*, *R. schneideri*, and *R. veredas* (Maciel et al., 2007). All species of this group occur in Brazil, except *R. poeppigii*, which occurs on the Andes slopes of Ecuador, Peru, and Bolivia (Frost, 2009). *Rhinella jimi*, distributed in northeastern Brazil, is closely related to *R. schneideri* from which it is distinguished by the combination of the following characters: presence of forearm gland, an external gland on feet, and gland conglomerates on both sides of the cloaca (Stevaux, 2002).

The tadpoles of *R. arenanum*, *R. cerradensis*, *R. icterica*, *R. marina*, *R. rubescens*, and *R. schneideri* have already been described (Kenny, 1969; Cei, 1980; Ford and Scott, 1996; Eterovick and Sazima, 1999; Rossa-Feres and Nomura, 2005; Maciel et al., 2007), no information is available about the tadpoles of *R. achavali*, *R. jimi*, *R. poeppigii*, or *R. veredas* (Maneyro et al., 2004; Kwet et al., 2006; Brandão et al., 2007). Here we describe the tadpole of *R. jimi* and compare it to the described tadpoles of the *R. marina* species group.

Material and Methods

Tadpoles were collected between 24 and 31 January 2009 by L. F. Toledo and R. Ribeiro in Fernando de Noronha, Pernambuco, Brazil (3°50'S, 32°25'W; sea level). *Rhinella jimi* is the only species of *Rhinella* present in Fernando de Noronha (Toledo and Ribeiro, 2010). All tadpoles were preserved in 10 % formalin and deposited in the amphibian collection of the “Prof. Adão José Cardoso” Zoology Museum (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Collecting permit (17242-1) was provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

Tadpole description is based on 20 specimens (ZUEC 14806) in stages 28 and 29 *sensu* Gosner (1960). Fifteen measurements were taken on the preserved tadpoles following the terminology of Altig et al. (1998), Altig and McDiarmid (1999) and Altig (2007): body length, body height, body width, tail length, maximum tail height, tail muscle height, tail muscle width, total length, oral disc width, internarial distance, interorbital distance, eye diameter, nostril diameter, eye-nostril distance, and nostril-snout distance. Measurements were taken using a digital caliper (0.01 mm of precision).

Five tadpoles of *R. schneideri* (ZUEC 15777 – collected by M. Martins in 1973 in Campinas, São Paulo, Brazil) in stages 27 to 29 were analyzed in order to make comparisons with the tadpoles of *R. jimi*. Student's *t* test was used to compare eye and nostril diameters between the species.

Results

Description of the tadpole. Measurements of larvae in stages 28 and 29 are provided in Table 1. Body depressed (body height/body width = 0.83), oval in dorsal and ventral views, elliptical in lateral view; body length about 44 % (41-50 %) of total length. Snout oval in dorsal view and sloped in lateral view. Nostrils large, nearly oval, located dorsally, with the opening dorsolaterally directed, with a small projection on the rim; nearer the eyes than the snout (eye-nostril distance/nostril-snout distance = 0.85). Small dorsal eyes (eye diameter/body width = 0.18) dorsolaterally oriented. Interorbital distance about twice the internarial distance. Spiracle single and sinistral, opening located near midbody, directed posteriorly. Inner (or centripetal) wall totally fused to the body wall and longer than the external wall. Medial vent tube, attached to ventral fin, with opening oriented posteriorly. Bicolored tail musculature; dorsal fin about same height as ventral fin. Dorsal fin originates near the tail/body junction and ventral fin begins anterior to vent tube. Oral disc anteroventral, laterally emarginated. Triangular marginal papillae with wide dorsal and ventral gaps, a single row of marginal papillae begins on each side of dorsal gap and ends on each side of ventral gap, few scattered sub-marginal papillae are located near the teeth rows laterally in the oral disc. Two anterior rows of labial teeth, the second one with a wide medial gap; three posterior rows of labial teeth, the first one with a small gap in one of the individuals analyzed; labial tooth row formula (LTRF) 2(2)/3[1]. Narrow jaw sheaths with triangular serration; lower jaw sheath 'U'-shaped and upper jaw sheath arch-shaped with long lateral processes. Dark brown coloration in preservative, with translucent non-pigmented fins; the internal organs are visible in ventral view. A tadpole at stage 28 is illustrated in Figure 1.

Natural history notes. Rhinella jimi tadpole is exotrophic and benthic (*sensu* Altig and Johnston, 1989). Tadpoles were always observed along pond edges and were active during the day but never at night. They inhabit lentic water bodies and form dense aggregations. These aggregations may be classified as stationary according to Beiswenger (1975). These tadpoles remain on the water surface exposed to the sun, but sometimes they can dive down to a depth of 50 cm and hide in sandy ground or under dead leaves. They also dive when approached. No feeding was observed.

Comparisons with other species. Some characteristics of the tadpoles of the described species of *R. marina* species group are presented in Table 2. Tadpoles of *R. jimi* are similar to tadpoles of the other species of the *R. marina* group. *Rhinella jimi* is distinguished from *R. schneideri* by having proportionally (diameter/body length) larger eyes and nostrils ($t_{23} = 5.41$; $P < 0.001$; $t_{23} = 9.00$; $P < 0.001$, respectively). The analyzed specimens also differed in eye orientation, with the eyes of *R. jimi* tadpoles more dorsally oriented than in *R. schneideri*. However eye orientation can be modified during development of tadpoles, so this difference may be merely a result of differences in developmental traits among the observed individuals.

Discussion

Tadpoles of the *R. marina* group are very similar in shape and general characters. Eterovick and Sazima (1999) suggested that *R. rubescens* is more similar to *R. schneideri* and that *R. arenarum* is more similar to *R. icterica* based on body proportions and spiracle position. According to Maciel et al. (2007) the tadpoles of *R. cerradensis* resemble *R. rubescens* and *R.schneideri* in body proportions and spiracle position. *R. jimi* tadpoles also resemble *R. rubescens*, *R.schneideri*, and *R. cerradensis* in spiracle position.

Based on available data on the spiracle position it is possible to form two groups of species: the first composed of *R. arenarum*, *R. icterica*, and *R. marina* with the spiracle opening located posteriorly (Kenny, 1969; Cei, 1980; Ford and Scott, 1996); and the second composed of *R. rubescens*, *R. cerradensis*, and *R. jimi* with the spiracle positioned midbody (Eterovick and Sazima, 1999; Maciel et al., 2007). *Rhinella schneideri* seems to have variable spiracle position among the different populations studied by Rossa-Feres and Nomura (2005) (with posterior spiracle) and by Cei (1980) (with midbody spiracle). *Rhinella cerradensis* tadpoles can be distinguished from other tadpoles of the *R. marina* group by the absence of an external tube in the spiracle (Maciel et al., 2007). According to Eterovick and Sazima (1999), *R. rubescens* can be diagnosed by its snout beign slightly truncated in lateral view. Therefore, *R. jimi* tadpole can be diagnosed by the spiracle with external tube opening on midbody, the snout sloped in lateral view, and the eyes and nostrils proportionally larger than in *R. schneideri*.

As we lack a complete or well accepted phylogeny of the genus (or at least of the *R. marina* species group) we are not able to link the morphological similarities/dissimilarities observed to evolutinary relationships. However, the tadpoles of *R. jimi* were morphologically more similar to those of *R. schneideri* (than compared to the other species of the group) just as observed for post-metamorphic individuals (Stevaux, 2002). Therefore, our study corroborates this hypothesized close relationship.

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Table 1. Mean measurements, standard deviations and range (in mm) of twenty *Rhinella jimi* tadpoles in Stages 28-29 of Gosner (1960).

Characteristic	Mean ±SD	Range
Body length (BL)	7.77 ±0.48	6.72 - 8.66
Body height (BH)	4.46 ±0.34	3.36 - 5.07
Body width (BW)	5.36 ±0.38	4.26 - 6.01
Tail length (TAL)	9.78 ±0.64	8.77 - 10.99
Maximum tail height (MTH)	3.93 ±0.17	3.59 - 4.20
Tail muscle height (TMH)	1.50 ±0.13	1.30 - 1.73
Tail muscle width (TMW)	1.19 ±0.10	0.96 - 1.34
Total length (TL)	17.57 ±1.04	15.40 - 19.05
Oral disc width (ODW)	2.26 ±0.17	2.05 - 2.72
Internarial distance (IND)	1.11 ±0.07	1.00 - 1.29
Interorbital distance (IOD)	2.31 ±0.14	1.95 - 2.57
Eye diameter (ED)	0.98 ±0.07	0.84 - 1.16
Nostril diameter (ND)	0.51 ±0.05	0.43 - 0.62
Eye-nostril distance (END)	1.17 ±0.08	1.00 - 1.38
Nostril-snout distance (NSD)	1.37 ±0.11	1.18 - 1.56

Table 2. Characteristics of the described tadpoles of the species of *R. marina* group. LTRF: Labial tooth row formula; BL: Body length; TL: Total length. Stages are presented according to Gosner (1960).

Species	Stage	LTRF	BL/ TL	TL	Spiracle position	Locality	Reference
R. arenarum	37	2(2)/3	less than 50 %	30	posterior	-	Cei, 1980
R. cerradensis	40	2(2)/3(1)	45%	20.08	on midbody	Brasília (Brazil)	Maciel et al., 2007
R. icterica	37	2(2)/3(1)	-	35	posterior	-	Cei, 1980
R. jimi	28–29	2(2)/3[1]	44%	17.6	on midbody	Fernando de Noronha (Brazil)	Present study
R. marina	29*	2(2)/3	40%	23	posterior*	Trinidad	Kenny, 1969
R. marina	33–36	2(2)/3	42%	22.4	posterior*	Jalisco (Mexico)	Ford and Scott, 1996
R. rubescens	25	2(2)/3(1)	43%	23.7	on midbody	Serra do Cipó (Brazil)	Eterovick and Sazima, 1999
R. schneideri	37	2(2)/3(1)	slightly more than 50 %	35	on midbody	Argentina	Cei, 1980
R. schneideri	36–38	2(2)/3	-	24.3	posterior	Nova Itapirema (Brazil)	Rossa-Feres and Nomura, 2005

*Information inferred from the pictures presented in the original articles.

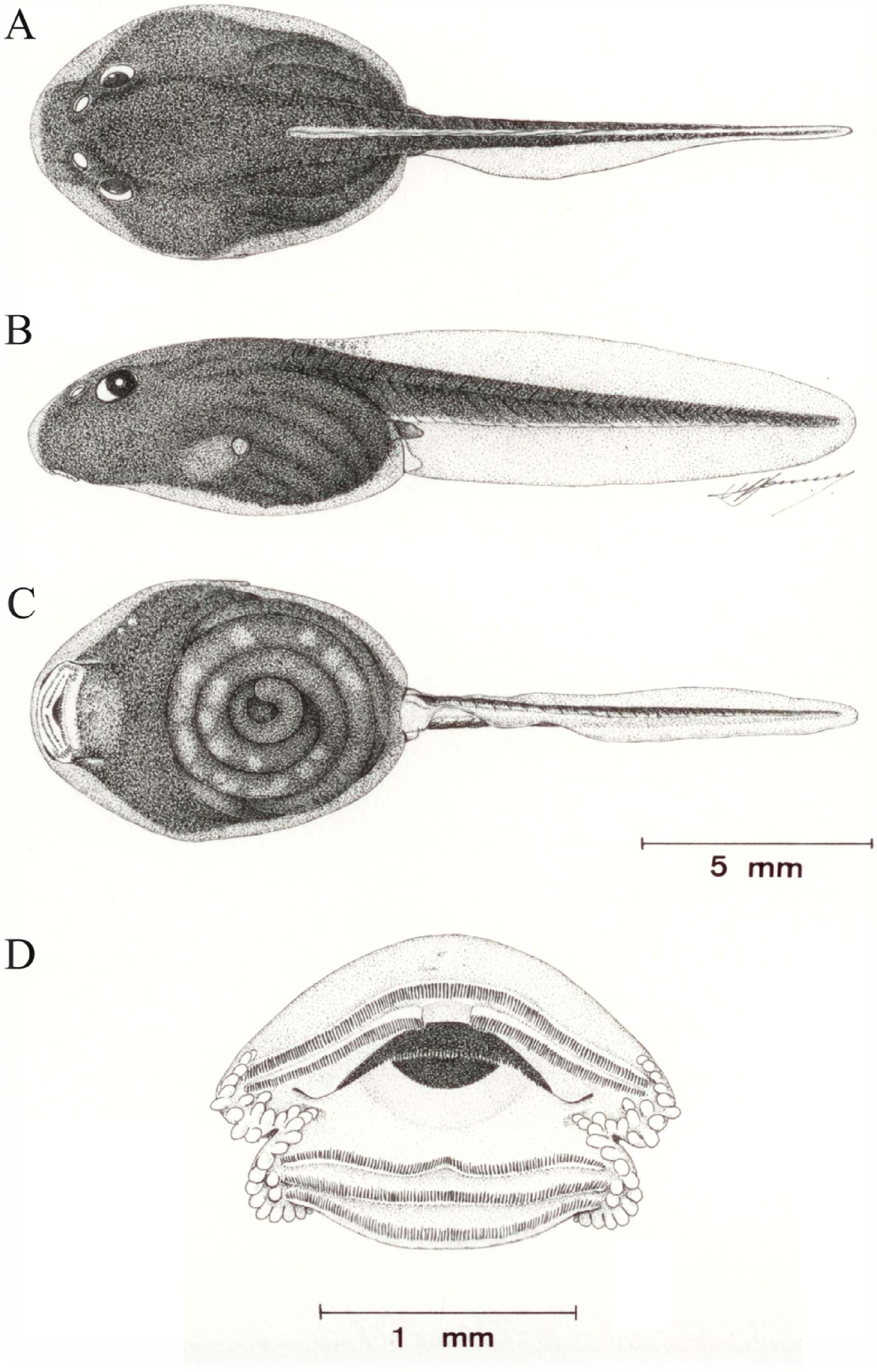

Figure 1. Tadpole of *Rhinella jimi*, stage 28: (A) dorsal, (B) lateral and (C) ventral views; (D) oral disc.

CAPÍTULO 2 - Deformed tadpoles in a Brazilian oceanic island and implications of oral deformities on their foraging behavior and food intake

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DEFORMED TADPOLES IN A BRAZILIAN OCEANIC ISLAND AND IMPLICATIONS OF ORAL DEFORMITIES ON THEIR FORAGING BEHAVIOR AND FOOD INTAKE

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Running Head: *Rhinella jimi* tadpole deformities

Key Words: amphibian deformities, introduced species, feeding

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Rhinella jimi (Anura, Bufonidae) was introduced in the archipelago of Fernando de Noronha (northeastern Brazil) about a hundred years ago and has established a large population since then. Almost half of the adult individuals of this population have external deformities, being one of the greatest amphibian malformation hotspot in the world, but tadpoles from this population have not previously been examined for deformities. Therefore, we evaluated the presence of deformities in tadpoles of that population, described the types of deformities, and identified possible handicaps of deformed tadpoles in foraging behavior and food intake. We run two experiments to test the influence of oral deformities in the foraging activity and feeding efficacy. The first evaluated how much time deformed tadpoles spent foraging in relation to normal ones. The second evaluated how much food deformed tadpoles ingested (during the same length of time) in relation to normal tadpoles. We found deformities in more than half of all tadpoles inspected, with deformities mostly involving abnormal dentition or mouth structure. Deformed tadpoles, when compared to normal individuals, forage less and have a lower foraging efficacy. We suggest that the reduced feeding fitness may imply in reduced growing rate, larger time spent until metamorphosis, higher predation risk, different body mass, size and morphology in metamorphs and adults. We also discussed possible causes of this elevate rate of deformity in this population.

Amphibians are declining worldwide due to multiple causes (Alford and Richards, 1999; Houlahan et al., 2000; Semlitsch, 2003; Lips et al., 2006; Collins and Crump, 2009) and the prevalence of deformed individuals, a possible factor contributing to amphibian declines, may be increasing in recent years (Cohen Jr., 2001; Blaustein and Johnson, 2003; Schoff et al., 2003; Lannoo, 2008). Malformations caused by mutations, developmental errors, and trauma are common, but they generally occur in 5 % or less of the individuals in a population (Blaustein and Johnson, 2003). However, recent observations reported higher rates of deformities (15–90 %) (Ouellet et al., 1997; Johnson et al., 2002; Blaustein and Johnson, 2003, Toledo and Ribeiro, 2009) caused by many different factors (Johnson et al., 1999; Ankley et al., 2000; Johnson et al., 2001; Ankley et al., 2002; Lajmanovich et al., 2003). Most of the available information about deformities in amphibians focuses on post-metamorphic individuals documenting mainly limb and digit deformities (Ouellet, 2000). Studies on oral deformities of larval anurans are scarce, but have demonstrated high rates of deformities (6-98 %) in some populations (Burger and Snodgrass, 2000; Drake et al., 2007) and that lack of keratin caused by a disease (chytridiomycosis) may affect feeding behavior and the total amount of food intake (Venesky, 2010). In spite of the lack of keratin in tooth rolls, the tadpoles of this last study did not present morphological malformations. Therefore, tadpoles with deformed oral discs have never been tested for their foraging fitness and its consequences.

In the Brazilian oceanic archipelago of Fernando de Noronha, there is a high rate (almost 45 % of the population) of malformed adult individuals of the introduced population of *Rhinella jimi* (Anura; Bufonidae) (Toledo and Ribeiro, 2009). This rate is greater than that observed in the natural range of the species, which varies from about 4 %

to 10 %. The causes of this high occurrence of deformed toads are yet unknown and some suggestions have been made (Toledo and Ribeiro, 2009). Toledo and Ribeiro (2009) examined only post-metamorphic individuals (mainly adults). Therefore it was not possible to determine if malformations occurred after or during the metamorphosis or if they were a developmental error already presented in the larval stage that persisted until the adult morphs. In this paper we examined the presence of malformations in tadpoles of the same population. Furthermore, if malformations were observed, we evaluated if they would affect the behavior and development of tadpoles, by testing the hypothesis that oral deformed tadpoles would be handicapped during feeding in relation to normal individuals.

MATERIAL AND METHODS

Field work.- *Rhinella jimi* tadpoles were collected in September 2009, April, May, and June 2010 by J. Tolledo, V. D. Fernandes, E. T. da Silva, M. P. Navarro, and L. F. Toledo in five different water bodies, in order to avoid sampling siblings, in the main island of Fernando de Noronha, state of Pernambuco, Brazil (3°50'S, 32°25'W; sea level). Specimens analyzed for malformations were fixed in a 7 % formalin solution and deposited as lots in the amphibian collection of the Museu de Zoologia “Prof. Adão J. Cardoso”, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil (ZUEC 16564–67, 16678–79, 16682, 16685–86, and 16688). Collecting permits were provided by Instituto Chico Mandes de Conservação da Biodiversidade – ICMBio (no. 19774–4).

Malformation evaluation.- All collected tadpoles were inspected with stereomicroscope and assigned to a larval stage (Gosner, 1960). Tadpoles were compared with those described by Tolledo and Toledo (2010). Oral deformities were classified according to Drake et al. (2007) except by the marginal papillae which were not examined. Oral deformities were assigned to seven regions of the oral apparatus (Fig. 1). Deformities not described by Drake et al. (2007) were here characterized.

Experiments.-To test the influence of oral deformities in foraging activity and efficacy we performed two experiments, similar to those reported by Venesky et al. (2010).

Experiment 1: Foraging Behavior

We compared foraging behavior of normal and abnormal tadpoles at three developmental stages: early (Gosner 26–29); intermediate (Gosner 30–34); and late (Gosner 35–40) (n = 10 for each stage in each condition – normal and abnormal - , but n = 12 for deformed tadpoles in early stage). Tadpoles were placed in15 x 10 cm plastic containers, supplied with 3 cm depth (450 mL) clean water and a stone encrusted with algae (which was so abundant in the stone that the tadpole was not limited in food availability). These stones were collected in the field after observations of natural feeding (Fig. 2A). After an acclimation period of two minutes, a naive experimenter (who did not know if the tadpole was deformed or not) observed the amount of time tadpoles spent foraging in a 12-minute trial. Each trial was divided into 20-second intervals and we recorded whether the tadpole was foraging or not during each interval. Then the proportion of time spent in foraging was calculated.

Experiment 2: Foraging Fitness

We tested whether oral deformities negatively affect tadpole foraging efficiency. The tadpoles were divided as in Experiment 1 (normal vs. deformed), n = 10 for each stage, but n = 9 and n = 8 for normal tadpoles in middle and late stages, respectively. Tadpoles were kept in 4 cm high and 5 cm diameter plastic containers (two tadpoles per recipient) with 40 mL clean water and were fasted for three days. The water was changed always we observed the presence of feces to avoid its ingestion. After that, tadpoles were placed in 700 mL plastic recipients (ten tadpoles per treatment) with stones collected in nature with algae (similar to experiment 1).

After three hours of foraging, tadpoles were killed and fixed in a 10 % formalin solution, and then they were dissected, having their gut removed. We measured with a caliper (to the nearest 0.01 mm) the total length of the gut and the portion which had feeding contents. The amount of filled gut during a three hour trial was considered a measure of foraging efficiency.

Data Analyses.- Linear regression analysis was utilized to compare prevalence of deformities with developmental stage, using developmental stage as the independent variable and the arcsine transformed data of proportion of malformed tadpoles as the dependent variable (Zar, 1999). We used a chi square analysis to compare deformity prevalence between larvae and adults, using data of Toledo and Ribeiro (2009) for adults and data of present study for tadpoles.

We used two two-way Analysis of Variance (ANOVA) to assess the impact of the presence of oral deformities and developmental stage on time spent foraging and on the efficiency of food intake, with deformity and developmental stage as the factors in both tests and the dependent variables being the arcsine square-root transformed data of proportion of time spent foraging and proportion of full gut, respectively. When null hypothesis was rejected, Tukey *post-hoc* test was applied (Zar, 1999).

RESULTS

A total of 413 tadpoles between stages 26 and 40 (Gosner, 1960) were analyzed, from which 217 (52.5 %) exhibited deformities to some degree. All of them presented oral deformities, two also had nostrils deformed, and ten also had deformed toes. A total of 997 oral deformities was observed, being 964 (96.7 %) in tooth rows and 33 (3.3 %) in jaw sheaths (Table 1). Oral deformities were of 19 types, of which nine have already been reported by Drake et al. (2007) and 10 were novel and described here (Table 1; Fig. 3A-N). Among the 72 tadpoles with toes fully differentiated (Altig and McDiarmid, 1999) (between stages 37 and 40) ten (13.9 %) presented reduced toes (Fig. 3O). One tadpole did not have the left nostril (Fig. 3P) and another tadpole had a shortened right nostril. The proportion of deformed tadpoles were not related to its developmental stage ($R^2 = 0.17$; $F_{1,13} = 3.85$; $P = 0.07$; Fig. 4). There were no differences between the amount of deformed adults and tadpoles ($\chi^2 = 2.86$; $P = 0.09$).

The foraging behavior experiment showed that oral deformities ($F_{1,56} = 4.06$, $P = 0.05$), developmental stage ($F_{2,56} = 20.83$, $P < 0.001$), and the interaction between these factors ($F_{2,56} = 6.30$, $P < 0.01$) affected the time spent foraging (Fig. 5). The foraging fitness experiment revealed a negative effect due to oral deformities ($F_{1,51} = 26.41$, $P < 0.001$), but no effects of developmental stage ($F_{2,51} = 0.13$, $P = 0.88$) and of the interaction between these factors ($F_{2,51} = 0.19$, $P = 0.83$) upon food intake by the tadpoles (Fig. 6).

DISCUSSION

The presence of abnormalities is common among natural populations, but the expected rate of deformities in amphibians, due to mutation, errors in development, and injury, is about 5 % or less (Blaustein and Johnson, 2003). The rate of abnormalities in *Rhinella jimi* tadpoles from Fernando de Noronha was more than 50 %, suggesting there are other than natural factors causing deformities in this population. Several factors are pointed out as possible causes of amphibian deformities, such as pesticide exposition (Bridges, 2000), trematode infection (Johnson et al., 1999), differential predation (Ballengée and Sessions, 2009), temperature during development (Bresler, 1954), and UVB radiation (Blaustein et al., 1997; Ankley et al., 1998). Besides these, inbreeding depression could also be a cause as it is associated with morphological malformations in other vertebrates (e.g. lizards - Olsson et al., 1996; fish - Afonso et al., 2000; and panthers - Mansfield and Land, 2002). However, this last cause has never been related to amphibian malformations (Williams et al., 2008).

There is no evidence of intensive usage of pesticides in Fernando de Noronha, since the agriculture practiced is basically for subsistence. But no water analysis was made to exclude this possibility yet. We searched for trematodes on toad musculature and no cyst was found (J. Tolledo and L.F. Toledo, person. obs.). Differential predation does not seem to be the case either, since it would not explain oral deformities, and we are not aware of any case in which predators caused oral deformities in tadpoles. Elevated temperatures during development and high UVB radiation may be explanations for oral deformities in amphibian species of temperate climate zone (Bresler, 1954; Blaustein et al., 1997). However, *Rhinella jimi* is a tropical species, originally occurring in northeastern Brazil (Stevaux, 2002; Frost, 2011), in temperatures and UVB radiation similar to those observed in Fernando de Noronha. We suggest that this is not the case either, but indicate thermal and UVB radiation experiments to confirm our suggestion. Inbreeding depression was not tested in the present study, but there is no evidence to exclude it. Therefore, we suggest that inbreeding depression is quite a plausible possibility, since we have evidence that only few individuals of the species were introduced in the archipelago and this population is isolated, increasing the chances of recurrent inbreeding (Frankham, 1997, 1998). Salinity to which these animals are exposed is another physiological stressor that may be present. Some small water bodies where toads breed are in contact with the ocean and we even saw some tadpoles swimming in the ocean near the encounter of the rivulets and the sea (Fig. 2B-C). Adults were also seen sheltering (Fig. 2D) and moving in the sand of beaches, undescribed micro-habitats for this species. As inbreeding, salinity has never been confirmed as one putative cause of amphibian malformation. Another explanation suggested by Toledo and Ribeiro (2009) to the high rate of deformities among *Rhinella jimi* population from Fernando de Noronha is that this rate could be natural. However, malformed toads are not removed by predators, as it would happen in the mainland population, because of the lack of a complete assemblage of predators in the island.

Tadpole developmental stage was not correlated with the prevalence of deformed individuals in the population. This result contrasts with that of other study (Burger and Snodgrass, 2000), suggesting that the mortality of deformed tadpoles should be similar to that of normal ones. Alternatively, we can not exclude the possibility that mortality in deformed tadpoles is higher, but there is an agent (e.g., toxins in the water) inducing abnormality over the time. The lack of differential mortality could be explained due to the lack of a structured predator community in Fernando de Noronha, as *R. jimi* was introduced in the archipelago, but not necessarily its complete and natural predator assemblage.

Although tadpole malformations are different from those observed in post-metamorphic individuals, the prevalence of deformity in tadpoles (52.5 %; present study) and adults (44.6 %; Toledo and Ribeiro, 2009) are not statistically different. This needs to be further investigated, to determine if deformities may originate in the initial stages of development and persists in the post-metamorphic forms, or if tadpole and adult deformities are independent.

The negative effect caused by malformations in feeding presented here do not result necessarily in higher mortality, but can affect the time of development and the size at metamorphose (Travis, 1984). A smaller body mass could expose them to a greater predation risk during the transition between aquatic and terrestrial environments, one of the most risky life stages for anurans (see Toledo, 2005). However, differential mortality does not seem to occur in this population (as suggested by the lack of difference in malformation prevalence across developmental stages) and we suggest three possible explanations: i) the predator assemblage in the island is deficient and the existent predators do not select for the handicapped tadpoles; ii) algae and other food items are so abundant in the water bodies that it is unlikely to exist resource competition leading to death of the malformed tadpoles; iii) and there are no negative fitness effects of the smaller size at metamorphosis or later metamorphosis; however we do not have data or observations to confirm this last hypothesis.

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Table 1. Types of malformations, number and percentage of deformed *Rhinella jimi* tadpoles (out of the 413 individuals sampled) from the Fernando de Noronha archipelago, Brazil. An asterisk "*" indicates deformities not described by Drake et al. (2007).

	Code	**Type of malformation**	**Deformed tadpoles**
Jaw sheath	JS1	Breaks, gaps, or other deformities in the cutting edge	10 (2.42 %)
	JS2	Breaks, gaps, or other deformities in the base of the jaw sheath	01 (0.24 %)
	JS3	Lack of keratinization in the jaw sheath	15 (3.63 %)
	JS4	Lack of jaw sheath*	02 (0.48 %)
	JS5	Lack of part of the jaw sheath*	03 (0.73 %)
	JS6	Left border of the inferior jaw sheath touching the midle of the superior jaw sheath*	01 (0.24 %)
Tooth rows	TR1	Missing teeth	18 (4.36 %)
	TR2	Missing teeth with disrupted supporting tissue	171 (41.40 %)
	TR3	Duplication of teeth (e.g.,double row, circular arrangement)	09 (2.18 %)
	TR4	Stunted teeth	91 (22.03 %)
	TR5	Intersecting tooth rows	25 (6.05 %)
	TR6	Puckering (sharp convolutions) of tooth rows	16 (3.87 %)
	TR7	Missing tooth row*	87 (21.07 %)
	TR8	Abnormal shape in tooth rows*	09 (2.18 %)
	TR9	Tooth row directed antero-posteriorly*	16 (3.87 %)
	TR10	Two or three tooth rows fused into one row*	30 (7.26 %)
	TR11	Not aligned tooth row*	01 (0.24 %)
	TR12	Short tooth row*	28 (6.78 %)
	TR13	Tooth row divided into two not aligned rows*	16 (3.87 %)

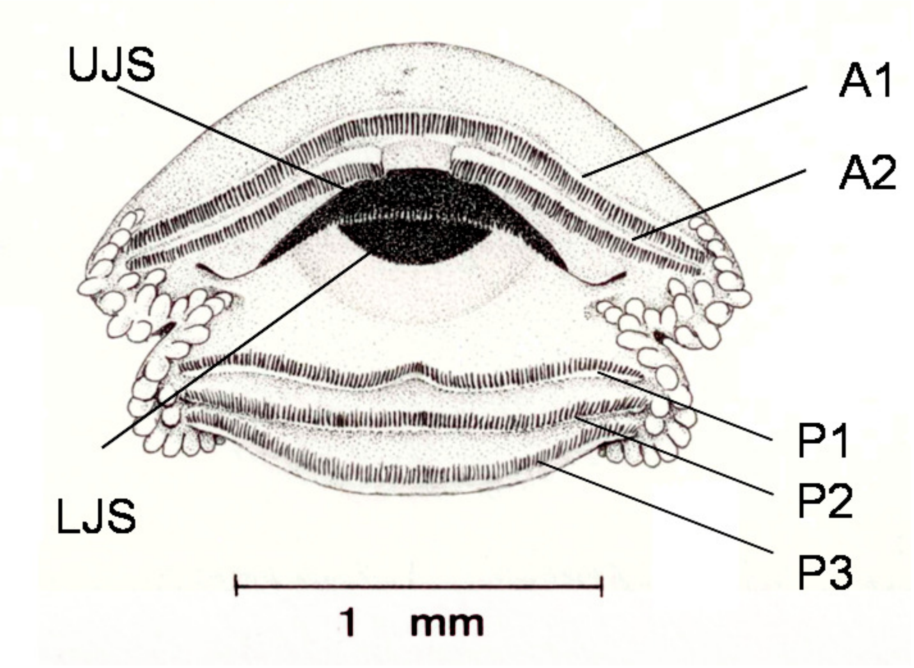

Figure 1. Oral apparatus of a normal *Rhinella jimi* tadpole, indicating the seven regions evaluated for deformities: A 1-2: anterior tooth rows 1 and 2; P 1-3: posterior tooth rows 1, 2 and 3; UJS: upper jaw sheath; LJS: lower jaw sheath. Modified from Tolledo and Toledo (2010).

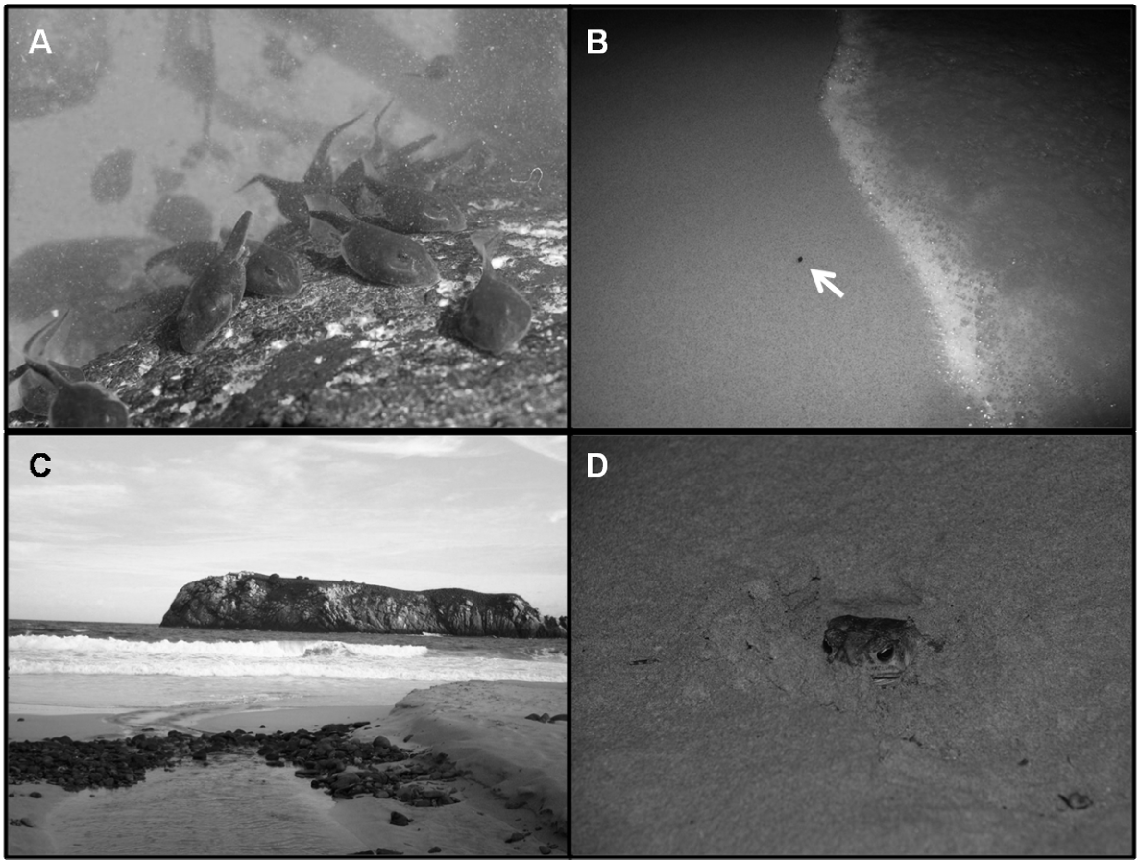

Figure 2. A: *Rhinella jimi* tadpoles feeding on algae incrusted in a stone; B: a tadpole (indicated by an arrow) swimming in the encounter of a rivulet with the sea at Praia do Leão; C: the encounter of Praia do Leão rivulet with the sea; and D: adult male *R. jimi* sheltering in the sand at Praia do Leão. All in the Fernando de Noronha archipelago, northeastern Brazil.

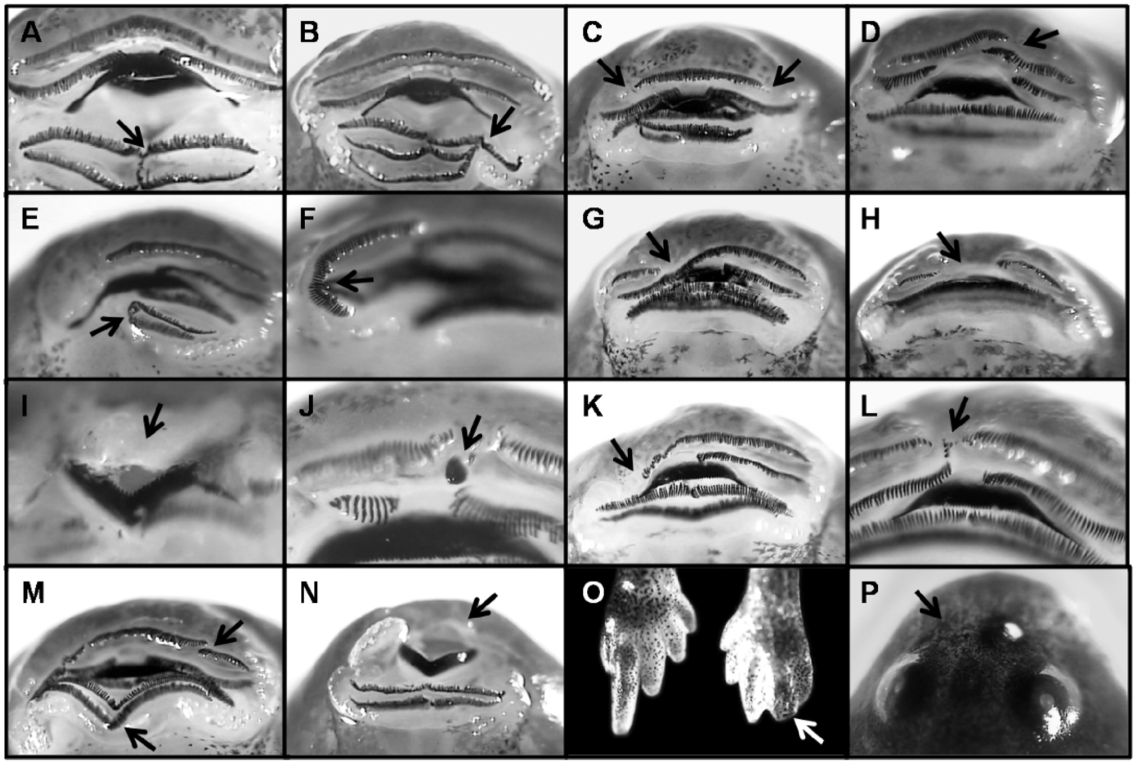

Figure 3. Deformities registered in *Rhinella jimi* tadpoles of Fernando de Noronha. A: Intersecting tooth rows and tooth row directed antero-posteriorly in P1-2 (TR5-9); B: Three tooth rows fused into one row in P1-2-3 (TR10); C: Short tooth row in A1 (TR12); D: Tooth row divided into two not aligned rows in A1 (TR13); E: Puckering of tooth rows and two tooth rows fused into one row in P2-3 (TR 6-10); F: Puckering of tooth row in A1 (TR6); G: Intersecting tooth rows in A1-2 (TR5); H: Missing teeth with disrupted supporting tissue in A1 (TR2); I: Lack of keratinization in the jaw sheath in UJS (JS3); J: Missing teeth with disrupted supporting tissue (TR2) with an abnormal keratinized structure in A1; K: Intersecting tooth rows in A1-2 (TR5) and Short tooth row in A2 (TR12); L: Tooth row directed antero-posteriorly in A1 (TR9); M: Tooth row divided into two not aligned rows in A1 (TR13) and Abnormal shape in tooth rows P2-3 (TR8); N: Missing tooth rows A1-2 (TR7) and Lack of upper jaw sheath (JS4); O: left foot with shorter toe IV; P: absence of the left nostril. Codes are in agreement with table 1.

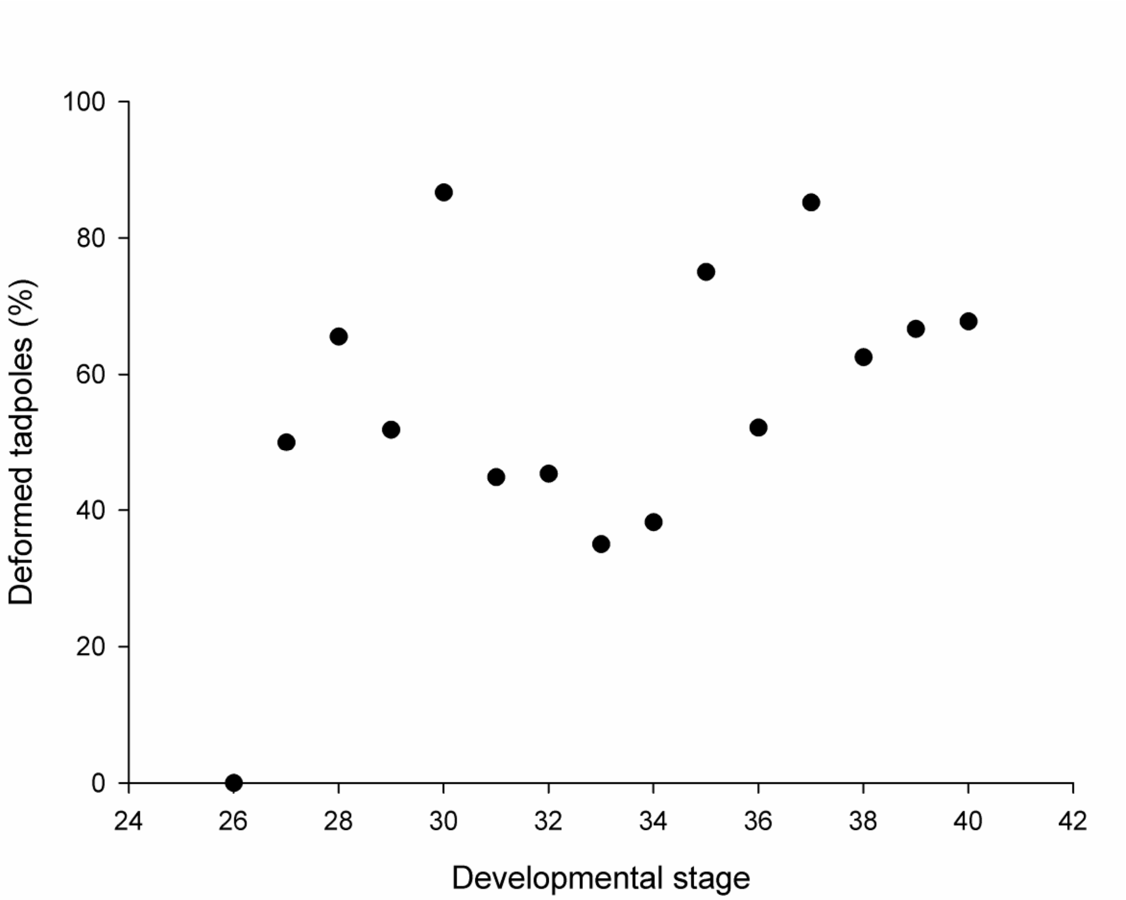

Figure 4. Percentage of deformed tadpoles of *Rhinella jimi* from Fernando de Noronha in each stage of Gosner (1960).

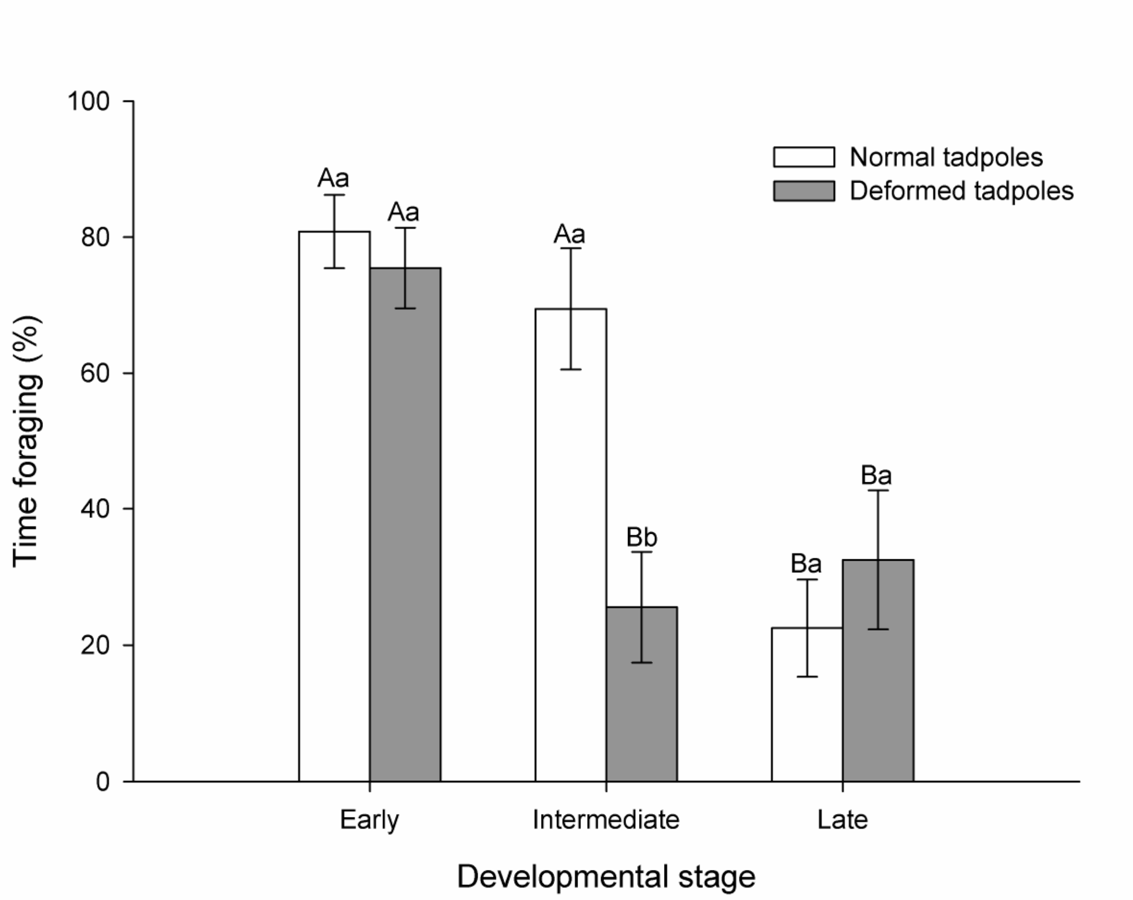

Figure 5. Percentage of time spent foraging by normal and deformed *Rhinella jimi* tadpoles of Fernando de Noronha archipelago, Brazil, in three different stages of development. Means and standard error bars. Early stages (26-29 of Gosner, 1960); intermediate stages (30-34 of Gosner 1960); and late stages (35-40 of Gosner 1960). Different uppercase represent differences between developmental stages and different lowercase represent differences between normal and deformed tadpoles as indicated by Tukey test at 5 %.

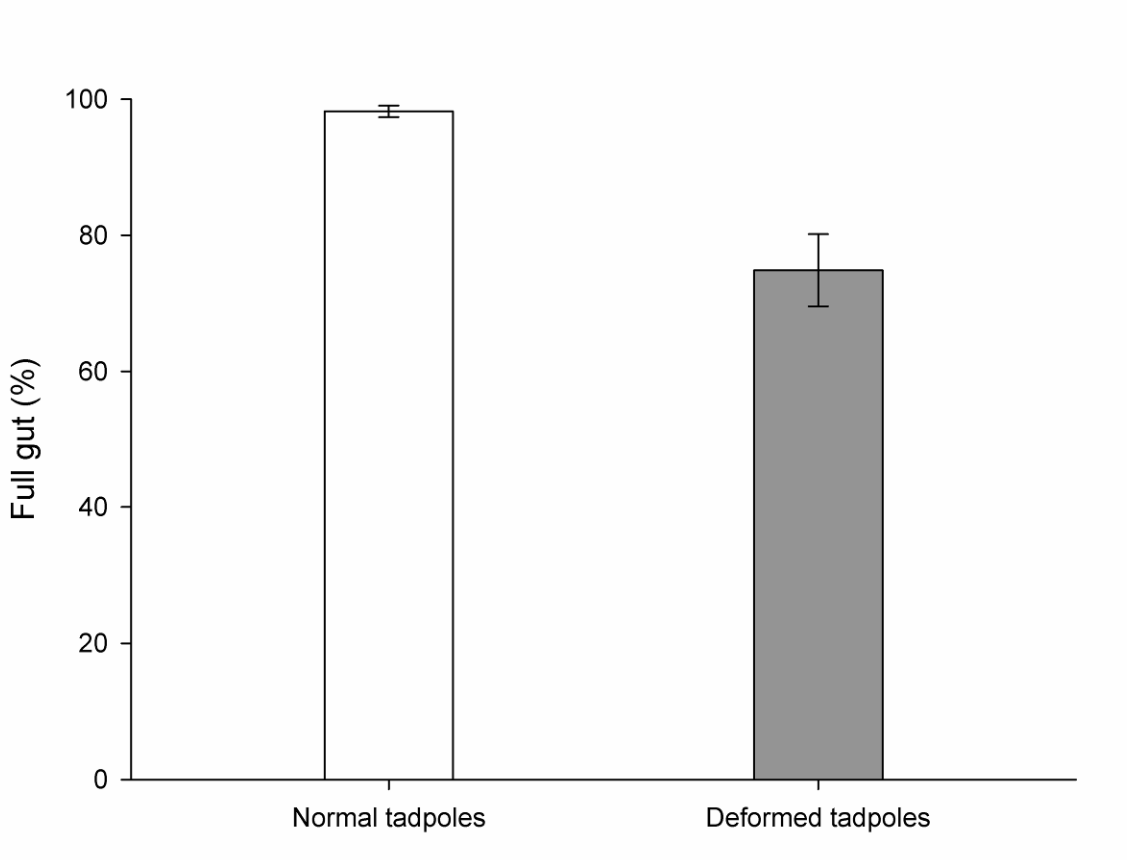

Figure 6. Percentage of gut with food content after a three hour trial of feeding in normal and deformed tadpoles of *Rhinella jimi* from Fernando de Noronha. Means and standard error bars.

CAPÍTULO 3 - Is the diet of an invasive population of *Rhinella jimi* (Anura, Bufonidae) affected by eye malformations?

JULIA TOLLEDO, RICARDO RIBEIRO & LUÍS FELIPE TOLEDO

JOURNAL OF HERPETOLOGY

LRH: J. Tolledo et al.

RRH: *Rhinella jimi* diet

Is the diet of an invasive population of *Rhinella jimi* (Anura, Bufonidae) affected by eye malformations?

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Key words: Amphibian deformities, introduced species, diet

Abstract. We studied the diet of an invasive species of toad on Fernando de Noronha, Brazil. *Rhinella jimi* was introduced to this island about 100 years ago. Almost half of the individuals from this large population are deformed. Many are blind or only have one functional eye. To elucidate the diet of this population we conducted a stomach content analysis, comparing deformed and non-deformed toads. We found 28 types of prey, indicating that *R. jimi* is a generalist predator. Among prey items, we highlight *Trachylepis atlantica*, a lizard endemic in Fernando de Noronha and *Johngarthia lagostoma*, a crab endemic in some oceanic islands that is endangered in Brazil. The diet of non-deformed toads was dominated by ants and beetles, whereas half-blind toads ate a large proportion of Diplopoda. We did not find any prey items in the stomachs of blind toads. To understand the diet of blind toads, we conducted dietary analyses using stable isotopes. Stable isotopes data did not differ statistically between toad groups, but showed a trend of deformed toads being enriched in heavier isotopes (^{13}C and ^{15}N), which may reflect the difficulty of these toads in finding food.

Introduced species, including invasive anurans, can change ecosystem processes by predation, competition, hybridization, or by acting as a vector for diseases that affect native species (Doody et al., 2009; Wittenberg and Cock, 2001). This is even more important in islands where there are several endemic and endangered species, and where the vulnerability to invasions is greater (Lowe et al., 2000), such as Fernando de Noronha, Brazil. Studying the diet of invasive species is important for identifying potential negative effects on native communities.

Rhinella jimi was introduced to Fernando de Noronha about 100 years ago, and the current population is large and spread over the main island. It is a predator that presents some natural history characteristics that help make it a successful invader, including large clutch size (Tolledo and Toledo, unpubl. data), high dispersal rates, and high tolerance to shifts in the environmental conditions (Duellman and Trueb, 1994).

Almost half of the adults and tadpoles of this alien population present external abnormalities (Toledo and Ribeiro, 2009; Tolledo et al., in prep.) and about 20 % of deformed adults have eye malformations. Some are blind and others are half-blind, presenting just one functional eye (Toledo and Ribeiro, 2009). As the foraging strategy of bufonids is often based on active search for preys (Toft, 1980; 1981) the diet of normal, blind, and half-blind toads may differ. However, the diet of *Rhinella jimi* in Fernando de Noronha may also be affected by an intrinsic characteristic of that population. We conducted analyses of stomach contents, predicting a diet similar to that of other bufonids, consisting mostly of ants and beetles (Parmelee, 1999). We predicted that *R. jimi* from Fernando de Noronha with vision problems would differ in prey consumption, since they would need to use different strategies to locate prey. We also evaluated diet by means of quantifying stable isotope levels. This method evaluates diet over a longer time (Araújo et al., 2007; 2009).

Materials and Methods

Field sampling

Collecting was carried out on the archipelago of Fernando de Noronha, Brazil, during January 2009 and May 2010. We collected 161 toads (76 adult males, 47 adult females, and 38 juveniles), of which 143 were normal, 14 half blind, and 4 blind, on the main island of the archipelago. We euthanized the toads with a lethal dose of thiopental on the same night of collection, removed the stomachs, and fixed them in 10 % formalin. The toes of an additional 18 *R. jimi* (6 normal, 6 blind, and 6 half blind toads) were removed and frozen for stable isotope analysis.

Stomach contents

Stomach contents were identified in the lab under a stereomicroscope. The items were identified to the level of order or better. We calculated the volume of each category of prey found in each stomach by means of alcohol displacement method. We used the index of relative importance (IRI; Pinkas et al., 1971) to describe the importance of each prey type, combining data on number, volume, and occurrence of preys. It was calculated by the formula: $IRI_t = (PO_t) \times (PI_t + PV_t)$. Where POt is the percentage of occurrence (100 x number of stomachs contained t item / total number of stomachs), PIt is the percentage of individuals (100 x total number of individuals of t in all stomachs/total number of individuals of all taxa in all stomachs), and PVt is the percentage of volume (100 x total volume of individuals of t in all stomachs/total volume of all taxa in all stomachs).

We analyzed the trophic diversity by standardized Simpson's reciprocal index: B/n, where $B = 1/ \Sigma P_i^2$, where pi is the proportion of the i^{th} prey item in the sample, and n is the total number of different resources (Pianka, 1973). The dietary overlap among groups was evaluated using the Pianka index (Pianka, 1973): $Ojk = Okj = [\Sigma (pij \times pik)] / (\Sigma pij^2 \times pik^2)^{0.5}$, where pij and pik are the proportion of the i resource used by the j and k categories of toads.

Stable isotopes

The previously frozen fingers were dried in a greenhouse at 60 ºC for 48h. After that, they were triturated and weighted, and approximately 1mg was placed in specific capsules for analysis. The relative abundances of ^{13}C and ^{15}N were determined at the Centro de Energia Nuclear, Agricultura, Universidade de São Paulo (CENA/USP), Brazil. Samples were analyzed in a Micromass 602E mass spectrometer (Finnegan Mat, Bremen, Germany).

Statistics

We used t-tests to compare number and volume of prey between half blind and normal toads (blind toads in our sample contained no food items). Changes on diets among groups were evaluated by *G*-tests using IRIs. We compared isotopic composition between normal, half blind, and blind toads using ANOVAs. We tested homogeneity of variances and normality prior to performing the analyses transformed the data when necessary. Differences were considered significant when $P < 0.05$ (Zar, 1999).

Results

Eighty six normal (62%), eight half blind (57%), and none of the blind toads presented stomach contents. We identified 28 prey categories, 27 of them invertebrates and one vertebrate (the endemic lizard *Trachylepis atlantica*; Table 1). Ants (Hymenoptera) and Coleoptera occurred in a large proportion of stomachs in both normal and half-blind toads. All prey categories were present in at least one normal toad stomach, and 14 of them were observed in half-blind toad stomachs. The number and volume of prey items per stomach were not significantly different between normal and half blind toads (number: $t_{95} = -1.27$, $P = 0.21$; volume: $t_{95} = -1.64$, $P = 0.10$; Fig. 1). However, the diet composition, considering the IRI, showed significant difference among the two toad classes ($G = 95.4$, df = 27, $p < 0.001$; Fig. 2). The trophic diversity was 0.12 to normal toads and 0.31 to half blind toads, and the trophic niche overlap between them was 0.35.

Normal toads contained large numbers of Coleoptera and ants, whereas half-blind toads had more termites (Isoptera) and Diplopoda. Normal toads also had a greater relative volume of Coleoptera and pupae of *Musca* (Diptera), whereas half-blind toads contained more Diplopoda and Coleoptera. All toads primarily fed on fast-moving organisms, but half-blind individuals also contained a large proportion of slow-moving prey (Fig. 3).

Relative frequencies of stable isotopes did not differ between groups (δ^{15}N: $F_{2,15} = 1.63$; $P = 0.23$; δ^{13}C: $F_{2,15} = 1.55$; $P = 0.24$; Fig. 4), but the difference was marginally significant (δ^{15}N: $t = -1.86$; $P = 0.08$; δ^{13}C: $t = -1.81$; $P = 0.09$) when comparing normal and all deformed toads combined. The trend observed was of blind and half blind toads being higher in ^{13}C and ^{15}N compared to normal ones.

Discussion

Bufonids are considered generalist predators (Duellman and Trueb, 1994). Toads are active hunters (*sensu* Pianka, 1966) and are able to find a wide range of prey types. They are opportunistic predators, feeding on almost every prey they can swallow (see

Boland, 2004). The broad diet of *R. jimi* is similar to what has been found in other species of the *R. marina* group (Sabagh and Carvalho-e-Silva, 2008; Quiroga et al., 2009; Issacs and Hoyos, 2010; Batista et al., 2011). Two prey species recorded in *R. jimi* from Fernando de Noronha deserve special attention. The crab *Johngarthia lagostoma* (Decapoda, Gecarcinidae) is endemic to oceanic islands (Ascension Island, belonging to England, and Trindade, Fernando de Noronha, and Atol das Rocas, belonging to Brazil; Hartnoll et al., 2006). It is considered threatened in Brazil as a result of habitat loss, touristic pressure, and other anthropogenic threats (Machado et al., 2008). The skink *Trachylepis atlantica* (Squamata, Scincidae) is endemic to Fernando de Noronha, the most western geographic record for the genus (Mausfeld et al., 2002). The predation of such endemic and endangered fauna highlights the possible negative impacts of this and other human-introduced species on the biodiversity of oceanic islands.

As none of the blind toads were found with contents in their stomachs, it is possible that they feed less frequently than normal individuals, hindered by the lack of vision, affecting directly their predation fitness. These deformed toads may present different strategies than normal ones to acquire food, and this may be determinant in the frequency of food acquisition by them and in the prey types consumed (e.g. fast-moving preys, which are more difficult to capture, were more common in the stomachs of normal toads). The trophic diversity was low for both toad groups, and was greater in half blind toads, what can be explained by the higher evenness among IRI of different prey types consumed by these toads. The value of niche overlap between half blind and normal toads was low (0.35 in a scale that varies between 0 and 1), corroborating the differences in diet composition.

The proportion of ^{15}N in tissues normally reflects trophic relations and increases with trophic level (Minagawa and Wada, 1984), suggesting that deformed toads feed on more carnivorous prey than so normal toads. However, starving animals also show increased values of ^{15}N (Hobson et al., 1993), suggesting that deformed individuals may have low body condition. Half-blind toads contained a large number of Diplopoda, but this cannot explain the differences in δ^{15}N, because these invertebrates are typically saprophagous (Hopkin and Read, 1992) and low in ^{15}N (e.g. Scheu and Falca, 2000). We suggest that the difference observed in δ^{15}N reflects the difficult of eye-deformed toads in obtaining food.

Further evidence that the diet of normal toads differs from that of blind and half-blind toads comes from measurements of ^{13}C. Plants with C_3 metabolic pathways have lower proportions of ^{13}C than do C_4 plants (Smith and Epstein, 1971; Post, 2002), and this is reflected in the tissues of consumers. Blind and half-blind toads in our sample are part of food webs based more on C_4 plants than are normal toads, perhaps because of sensory-induced differences in foraging strategies.

The present study demonstrated differences in the diets of normal and deformed toads. Different diets may, in turn, influence on size, mass, and ultimately on reproductive success of malformed toads. Therefore, lack of an eye (or both eyes) affects several ecological features of amphibians.

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Table 1. Numeric frequency, volumetric frequency, and frequency of occurrence of different prey types found in the stomachs of normal and half blind toads (*Rhinella jimi*) from Fernando de Noronha.

		Numeric Frequency		Volumetric Frequency		Frequency of occurrence	
	Prey type	Normal	Half blind	Normal	Half blind	Normal	Half blind
INVERTEBRATES							
Insecta							
Blataria		0.20	-	3.09	0.00	10.11	-
Coleoptera							
Larvae	**SM**	0.95	-	1.52	0.00	13.48	-
Pupae	**M**	0.02	-	0.00	0.00	1.12	-
Adults	**FM**	30.94	3.60	46.99	19.51	69.66	50.00
Dermaptera		2.61	0.90	1.91	0.00	21.35	25.00
Diptera							
Larvae (Musca)	**SM**	8.79	5.41	13.21	0.00	17.98	12.50
Pupae (Musca)	**M**	3.71	0.90	7.81	0.00	15.73	12.50
Adults (Musca)	**FM**	1.65	-	4.55	0.00	12.36	-
Adults (Nematocera)	**FM**	0.06	-	0.28	0.00	4.49	-
Hemiptera	**FM**	0.16	4.50	0.51	9.76	6.74	25.00
Homoptera	**FM**	1.55	-	0.84	0.00	13.48	-
Hymenoptera							
Wasps	**FM**	0.75	1.35	2.98	2.44	8.99	25.00
Ants	**FM**	44.99	16.22	5.06	2.44	74.16	50.00
Isoptera	**FM**	0.14	36.94	0.06	4.88	4.49	12.50
Lepidoptera (larvae)	**SM**	0.33	-	0.79	0.00	8.99	-
Ortoptera	**FM**	0.06	-	0.28	0.00	4.49	-
Phasmida	**FM**	0.02	-	0.00	0.00	1.12	-
Molusca							
Gastropoda	**A**	0.23	-	0.62	0.00	2.25	-
Crustacea							
Amphipoda	**A**	1.10	-	0.51	0.00	1.12	-
Isopoda	**A**	0.02	0.45	0.00	2.44	1.12	12.50
Decapoda							
Ocypode spp.	**FM**	0.02	-	0.62	0.00	1.12	0.00
Gecarcinus lagostoma	**FM**	0.09	0.45	2.70	0.00	6.74	12.50
Chelicerata							
Acari	**FM**	0.29	2.70	0.06	0.00	3.37	12.50
Aranae	**FM**	0.19	0.90	0.06	7.32	8.99	12.50
Opiliones	**FM**	0.03	-	0.17	-	2.25	-
Miriapoda							
Chilopoda	**FM**	0.03	0.45	1.12	2.44	1.12	12.50
Diplopoda	**SM**	1.09	25.23	3.37	48.78	23.60	25.00
VERTEBRATES							
Reptilia							
Squamata							
Trachylepis maculata	**FM**	0.02	-	0.90	-	1.12	-

SM: slow motion; M: motionless; FM: fast motion; A: aquatic.

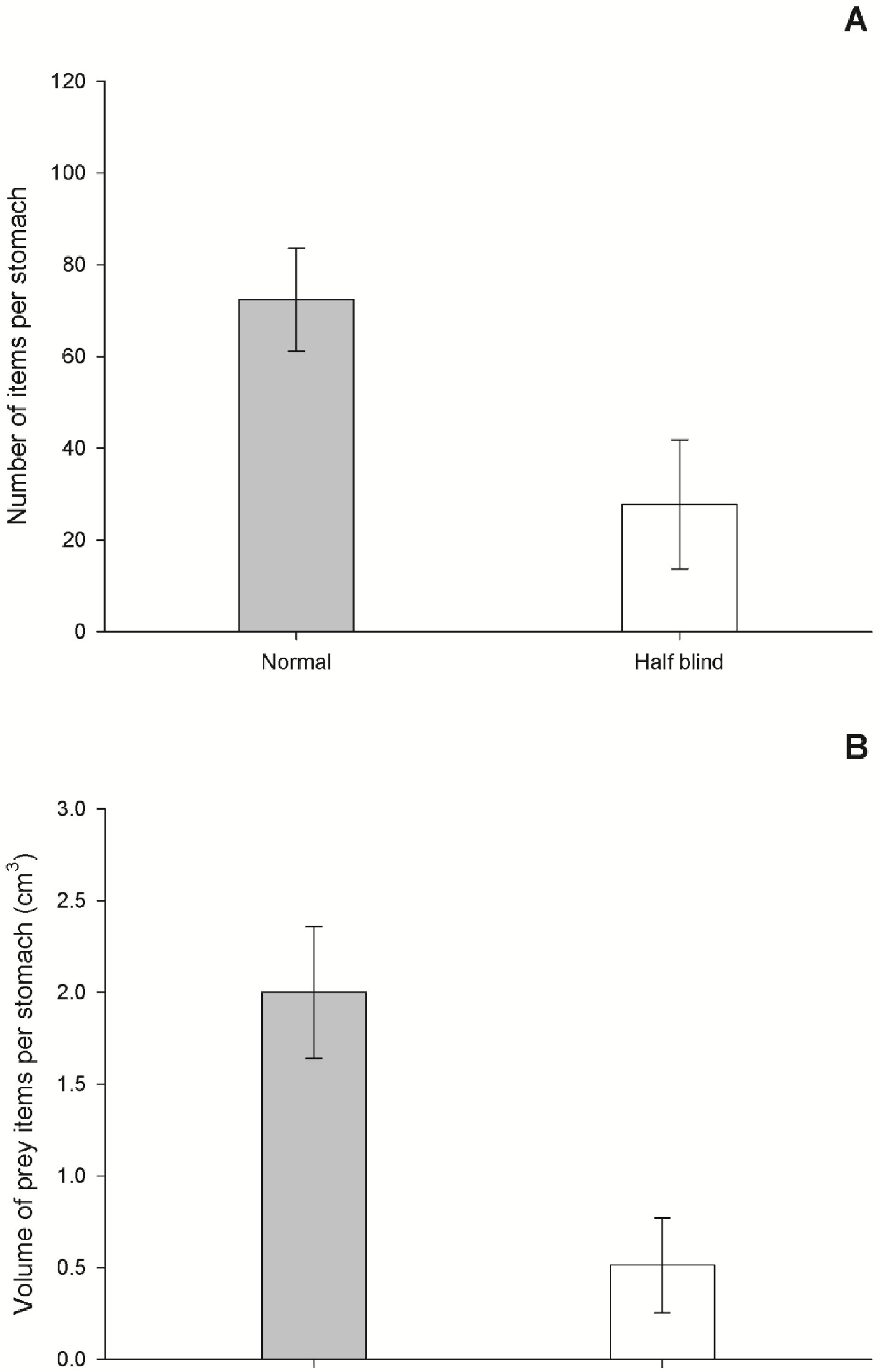

Figure 1. Number (A) and volume (B) of prey items present in the stomachs of normal and half blind individuals of *Rhinella jimi* toads from Fernando de Noronha, Brazil. Bars are standard errors.

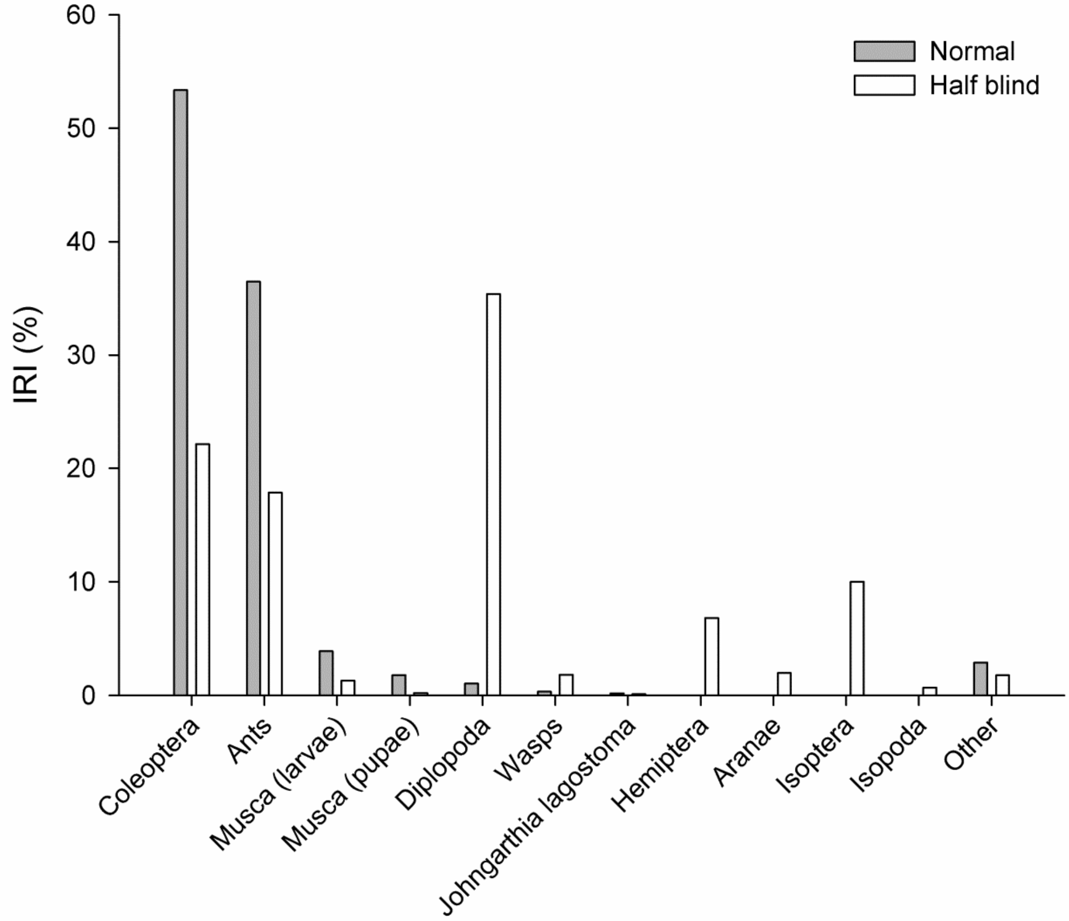

Figure 2. Proportion of index of relative importance (Pinkas et al., 1971) of each prey type found in the stomachs of normal and half blind toads (*Rhinella jimi*) from Fernando de Noronha, Brazil. The category "other" is the sum of all prey types with IRI lower than 1% in both predator types.

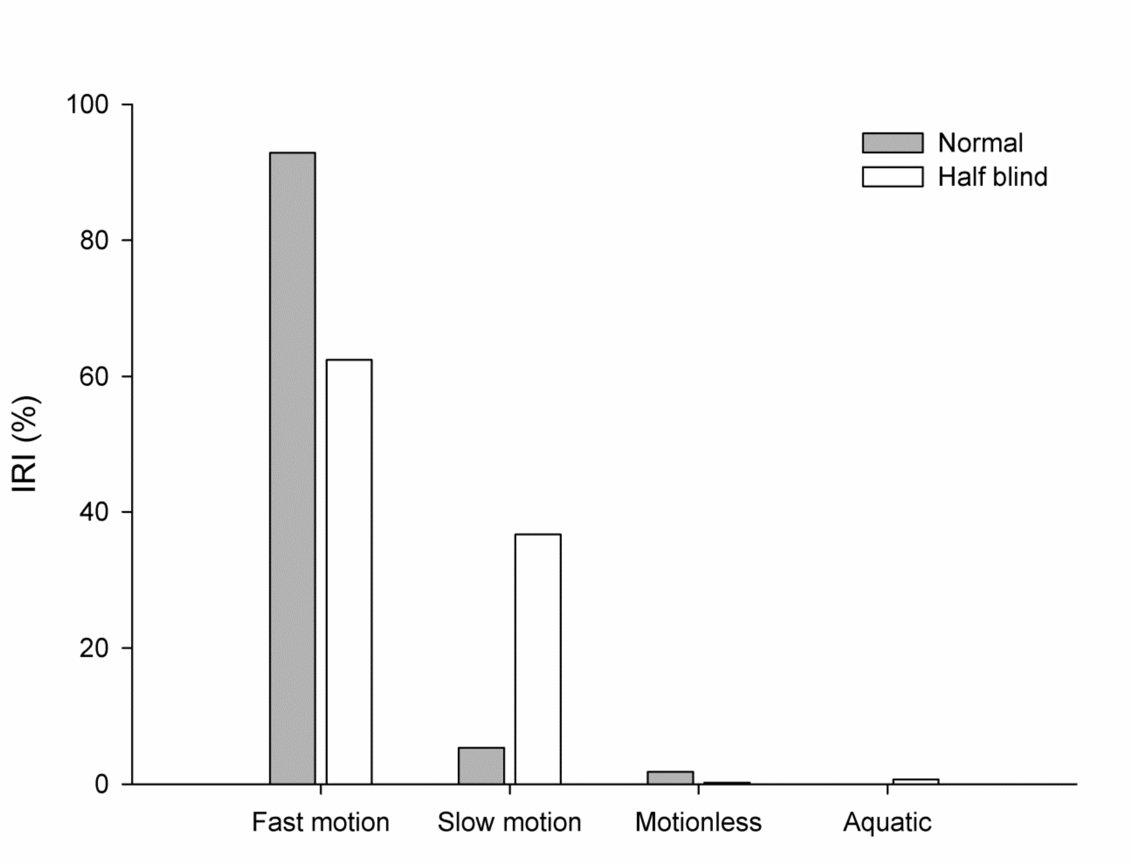

Figure 3. Numeric frequency of the different preys (classified according to their habits) registered in the stomachs of normal and half blind toads (*Rhinella jimi*) from Fernando de Noronha, Brazil.

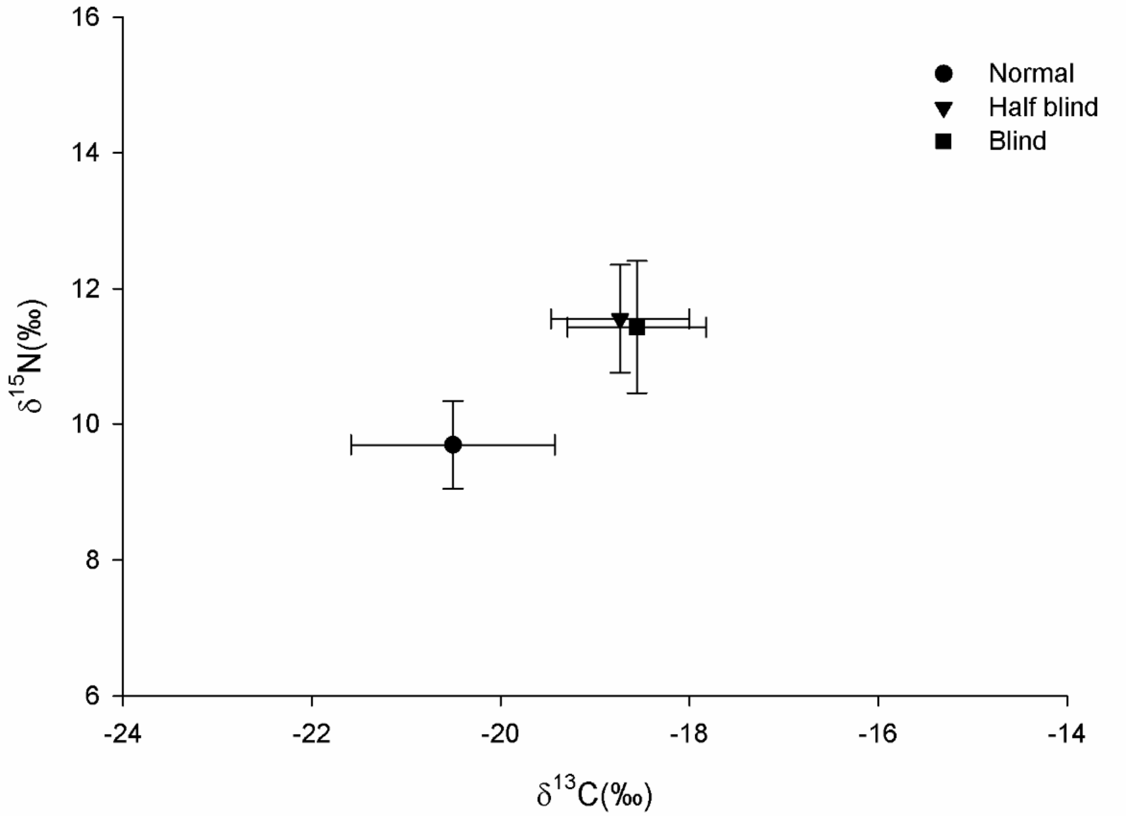

Figure 4. δ^{15}N and δ^{13}C signatures of normal, half blind, and blind toads (*Rhinella jimi*) from Fernando de Noronha, Brazil. Bars denote standard errors.

CAPÍTULO 4 - How do visual orientated toads feed when they are blind?

JULIA TOLLEDO, MILENA PRENDIN NAVARRO & LUÍS FELIPE TOLEDO

HOW DO VISUAL ORIENTATED TOADS FEED WHEN THEY ARE BLIND?

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ABSTRACT: Generally nocturnal, anurans developed extraordinary visual skills allowing precise locating and capturing their preys. Therefore, vision is expected to be crucial for their basic vital activities. In spite of this, we found an introduced population of common toads (Bufonidae: *Rhinella jimi*) of which many individuals are blind. For that reason, we investigated how do blind toads locate and capture their preys and analyzed some possible disadvantages of being blind. We found that blind toads made use of alternative predation strategies from normal ones: they sensed the preys with tact and exhibited a sit-and-wait foraging strategy, whereas normal toads located prays with vision and hunted them actively. Besides the different strategies, both groups (blind and normal) had similar prey capture success, similar adipose body tissue mass, and similar number of oocites produced per female. On the other hand, blind toads were smaller in size and lighter than normal toads of the same weight and size, respectively. Our study revealed an amazing behavioral plasticity to survive in an unusual and adverse condition, probably imposed by the artificial introduction of this population in an isolated oceanic Archipelago.

Key-words: Blindness; Deformities; Behavioral plasticity; Introduced species, Foraging strategy

Introduction

With few exceptions, such as *Xenohyla truncata* (Anura, Hylidae) that also feeds on fruits (Silva et al. 1989), anurans are exclusively carnivorous, feeding mainly or exclusively on small invertebrates (Duellman & Trueb 1994). To locate their preys, anurans may use olfactory (Alexander 1965; Shin and Dole 1979; Rossi 1983), auditory (Jaeger 1976), or tactile (Comer & Grobenstein 1981) cues. However, most of the species rely on their visual system for prey localization, identification, approach, and subjugation (Nishikawa 2000). Furthermore, anurans can modulate their feeding behavior in response to the visual analysis they make from their prey, increasing the feeding success (Anderson & Nishikawa 1996; Valdez & Nishikawa 1997).

Feeding success interferes directly in vital aspects of the individual’s biology, such as providing enough energy for the maintenance of its basal metabolic rates and growth (Schmidt-Nielsen 1996). Besides allowing its own persistence, feeding fitness may also be strictly correlated to the reproductive success. I.e., there is a high amount of energy demand during reproductive activities, such as the calling behavior in anurans, considered one of the most costly behaviors among ectothermic vertebrates (Prestwich et al. 1989; Wells & Taigen 1989; Gerhardt 1994), and egg production, which may consume almost half of the female caloric content (Smith 1976). Therefore, as more efficient in food acquisition and quality, more energy the individuals will be able to invest in reproduction (Schoener 1971; Lemon 1991; Wiersma &Verhulst 2005).

Besides this, body size and mass are directly correlated to reproductive success of males that are under female's selection (e.g., Wells 1977; 1979; 2007). Generally, females choose those males that are larger (e.g. *Anaxyrus quercicus* Wilbur et al. 1978; *Anaxyrus americanus* Howard 1988). Larger males tend to win physical combats as well (Shine 1979), which could be essential to defend a calling or egg laying site. Size and body mass also is correlated to females' success. For example, as larger the female is, larger the amount of eggs it may produce (Davies & Halliday 1977; Tejedo 1992; Duellman & Trueb 1994; Pombal & Haddad 2005; Wells, 2007). Hence, feeding fitness affects individuals size and mass, ultimately influencing in reproductive success.

In the Brazilian oceanic archipelago of Fernando de Noronha there are high rates of malformed individuals of the introduced toad *Rhinella jimi* (Anura; Bufonidae). The rates reach about 45 % of the post-metamorphic (adult) individuals (Toledo & Ribeiro 2009) and about 52 % of the tadpoles (J. Tolledo, personal observation). Among the adults, about 20 % of the malformation cases are related to eye deformities, such as anophthalmia, palpebrae malformation, and lack of colored iris (Toledo & Ribeiro 2009). Based on the fact that anurans rely mostly in visual cues for feeding (Nishikawa 2000) we investigated how toad blindness affect their feeding behavior and evaluated the possible fitness consequences of being blind (or partially blind, in cases of single eye anophthalmia). We hypothesized that blind toads would use alternative strategies, in relation to visual perception, to locate their preys. We also hypothesized that blind toads would be handicapped in relation to normal toads considering food intake, body mass, quantity of adipose body, and number of eggs produced per female. Finally, we expect that single eyed toads would present intermediate results when compared to normal and complete blind toads.

Methods

We realized experimental trials to observe how would blind toads locate and capture their preys in relation to normal toads. Therefore, 21 toads (10 blind and 11 normal) were collected in the archipelago of Fernando de Noronha, Pernambuco, Brazil. One eyed toads were not tested in this trial. They were kept in captivity and prevented of food for three days. After that, they were provided with four adult crickets (collected in the field in the same day of the experiment) and recorded with a digital video camera Sony HandyCam DCR-SR47 during eight hours overnight (from 11 p.m. to 7 a.m.). After trial each toad was released in the same place it was collected. We analyzed the videos with Picture Motion Browser (PMB – Sony). We adopted the methods of all occurrences sampling to qualify the behaviors and sequence sampling to quantify them (Altmann 1974; Lehner 1996; Del-Carlo 2004). We registered the number of attempts to capture the prey, how many were successful, how many times crickets touched the toad, and if the cricket touched or not the toad before each predation attempt.

Feeding trails run in a cardboard box (30 x 50 x 40 cm height), which had one of the walls made of glass, allowing film recording. Trails run in an acclimatized room (air temperature near 25 ºC) with artificial illumination.

To test the hypothesis that blind toads are handicapped in relation to normal ones in traits associated with alimentation, we collected blind, half blind (one eyed toads), and normal toads in Fernando de Noronha and euthanized them with a lethal injection of sodium thiopental solution in the brain through the Foramen Magnum. After that, we weighted, measured the snout-vent length (SVL), and did necropsies in adult toads to remove gonadal adipose tissue (of both sexes) and the female ovaries. Female ovaries were weighted and the number of eggs estimated. This estimation was as follows: we weighted the ovaries, removed a sub-sample of about 3 g from it, counted the oocytes in the sub-sample, and estimated the total number of oocytes in the ovaries. To measure the SVL we used a digital caliper to the nearest 0.1 mm. Weighting was made with field compact scales to the nearest 0.05 g.

To compare the number of attempts to capture the prey, and the proportion of successful attempts during the video trails, we did two t-tests, with independent variable being blindness and the dependent variable being number of attempts to capture prey and arcsine transformed data on the proportion of successful attempts (Zar 1999).

Data on physical conditions of blind, half blind and normal toads were analyzed using Analysis of Covariance (ANCOVA). We tested for differences in body mass, gonadal adipose tissue mass, and number of eggs produced between the three groups, considering size, body mass suppressed of adipose body mass and body mass suppressed of egg mass as covariables respectively.

Results

We observed four different behaviors for the predators (toads): (i) moving toward a prey; (ii) attempt to capture a prey by tongue projection; (iii) immobility; and (iv) removing the prey, when it was on the toad. For the preys (crickets) we observed three different behaviors: (i) to move in front of the predator; (ii) touch the predator; and (iii) escape from the predator. These behaviors followed an escalated order as presented in Figure 1.

Feeding behavior was different between blind and normal toads. Normal toads did behavior as active foragers using vision exclusively to locate their preys. Blind toads did behavior as sit-and-wait predators locating their preys by means of tactile stimuli exclusively. Crickets touched each blind toad 31.25 (± 32.90) times and each normal toad 0.64 (± 1.03) times in average. Of the 40 attempts to capture the insects by blind toads only one did not occurred after the cricket touched it. None of the 158 attempts of the normal toads to capture the crickets was after tactile stimuli with the cricket (Table 1).

Blind toads did less attempts to capture preys than normal ones ($t_{19} = -4.6$, $P < 0.001$, Fig 2A), but the proportion of successful attempts did not differ between blind and normal toads ($t_{16} = 1.39$, $P > 0.1$, Fig 2B).

Blind toads have smaller body mass in relation to their size than normal individuals, and half blind toads were intermediate ($r^2 = 0.71$, $P < 0.01$; $F_{2,235} = 3.47$, $P = 0.03$, Fig 3). The gonadal adipose tissue of adults and the total number of oocytes produced per female did not differ between blind, half blind, and normal toads ($F_{2,50} = 0.29$, $P > 0.05$ and $F_{2,15} = 2.49$, $P > 0.05$, respectively, Fig. 4).

When in captivity three blind toads ate their own skin. During this behavior, the toad remained motionless for a long time and then started to do some slow movements bringing the loose skin toward its mouth. After that, the toad slowly opens its mouth and begins to eat the skin.

Discussion

As we observed, predation strategies employed by normal and blind toads are opposite (active against sit-and-wait forager; visual against tactile cues). This behavioral plasticity is an amazing example of amphibian ability to adapt to adverse conditions, which could be deleterious for many vertebrates in natural systems. As observed in Fernando de Noronha, blind and other malformed toads are able to live, feed, and breed in equal numbers as normal toads (Toledo & Ribeiro 2009). However, we observed that blind toads may be handicapped in relation to normal ones in size and mass. It could be a consequence of the sit-and-wait plus the tactile sense strategy displayed by blind toads; as it may provide less meals than the active foraging plus visual sense strategy. In fact, we observed that blind toads have less stomach contents than half blind, which in turns presented less stomach contents then normal toads (J. Tolledo & L. F. Toledo, personal observation).

Body mass influences individual fitness in several ways. Usually smaller and lighter males mate fewer times than larger ones (Bastos & Haddad 1996) and they generally loose aggressive interactions, such as territorial fights, scramble for females and attempt to dislodge amplectant males, common behaviors among explosive breeders (Wilbur et al. 1978; Arak 1983; Bastos & Haddad 1996; Toledo & Haddad 2005). Smaller and lighter females are also handicapped since the body mass of a female seems to be positively correlated to egg production (Berven 1988; Bastos & Haddad 1996; Prado & Haddad 2005). In spite of this, our results did not indicate difference of reproductive effort between blind and normal females. Although the tactile feeding strategy may provide less and unselected food items, the sit-and-wait strategy may be less energetically costly for the toads. Therefore, blind toads may be lighter but it may not affect the reproductive effort at all.

As predicted by the optimal forage theory, animals should select the best diet based on associated energetic costs (Schoener 1979; MacArthur & Pianka 1966). Prey selection also depends on relative abundance of prey items in the habitat and on traits of each prey item, such as size, shape, motion direction and speed (Valdez & Nishikawa, 1997).

Bufonids are usually active hunters (wide foraging, *sensu* Pianka 1966), feeding mainly on small and motionless preys that they encounter during their foraging activity in relatively large areas (Toft 1980). Predators using tactile cues to locate preys are probably unable to perceive either prey abundance or morphological traits before capturing them (Duellman & Trueb 1994). As blind toads are not able to discriminate abundance, size, shape, and any other visually detectable traits of their preys, we suggest that they will eat any touched food item and are not able to select an optimal diet, as normal ones should be. In corroboration, we observed significant differences in the diet of normal and blind toads (Tolledo, Ribeiro & Toledo, unpublished data).

Moulting and eating the shed skin are common behaviors among amphibians (Duellman & Trueb 1994) and have been reported for many anuran species (Weldon et al. 1993), including species of *Rhinella marina* group, as *R. marina*, *R. arenarum* (Weldon et al. 1993), and *R. icterica* (Sabagh & Carvalho-e-Silva 2008). This phenomenon is not well studied and the causes and consequences of its occurrence are still unknown. The shed skin represents a loss of energy, unless it is consumed (Smith 1976). Some authors suggest that it can supply protein by reincorporating that present in the old skin (Weldon et al. 1993). For blind *Rhinella jimi* from Fernando de Noronha this can be an important way to save energy and protein, as these individuals have difficult in having food.

We suggest also that the capacity to perceive and react to tactile stimulus and to exhibit the sit-and-wait strategy were not acquired by blind toads, since the population has been introduced in the island about 100 years ago (Toledo & Ribeiro 2009), and, therefore, there was probably not enough time for such trait evolution. Thus, we suggest that all individuals in the population, or even of the species, are able to make use of these alternative strategies (see also Comer & Grobstein 1981). As we did not observe tactile hunting in normal toads, we also suggest that it should be disadvantageous in relation to visual hunting. This advantage would be in relation to food selection, individual feeding specialization, and feeding efficacy (e.g., hunting more preys in less time).

Anurans face severe modifications in its diet across life stages, many being herbivorous during larval stages and carnivorous after metamorphosis (Duellman & Trueb 1994). There have also been observed ontogenetic shifts in the diet, where juveniles feed on different prey items than adults (Rodrigues et al. 2004), sexual differences, where adult males feed on different items than adult females (Donnelly 1991; Brasileiro et al. 2010; Forti et al. 2011) or even individual specialization (Araújo et al. 2007; 2009). Therefore, anurans are largely plastic in its feeding ability and the present report exemplifies an extreme case of feeding plasticity within individuals of the same age and sex, but in different structural conditions.

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Table 1. Attempts to capture prey, successful attempts, and stimuli before attempts. Observed during each video trial with adults of *Rhinella jimi* from Fernando de Noronha.

	Toad	**Sex**	**Attempts**	**Successful attempts**	**Stimuli before attempts**
Blind	1	Male	11	1 (9.1 %)	tactile
	2	Male	10	1 (10 %)	tactile
	3	Male	0	0	-
	4	Male	5	2 (40 %)	tactile
	5	Male	1	1 (100 %)	tactile
	6	Male	9	1 (11.1 %)	tactile + unknown
	7	Male	2	0 (0 %)	tactile
	8	Male	0	0	-
	9	Male	2	1 (50 %)	tactile
	10	Male	0	0	-
Normal	1	Male	5	1 (20 %)	visual
	2	Female	17	1 (5.9 %)	visual
	3	-	9	1 (11.1 %)	visual
	4	Male	19	1 (5.3 %)	visual
	5	Male	6	4 (66.7 %)	visual
	6	Female	8	3 (37.5 %)	visual
	7	Male	18	3 (16.7 %)	visual
	8	Female	27	3 (11.1 %)	visual
	9	Male	17	4 (23.5 %)	visual
	10	-	20	4 (20 %)	visual
	11	Female	12	4 (33.3 %)	visual

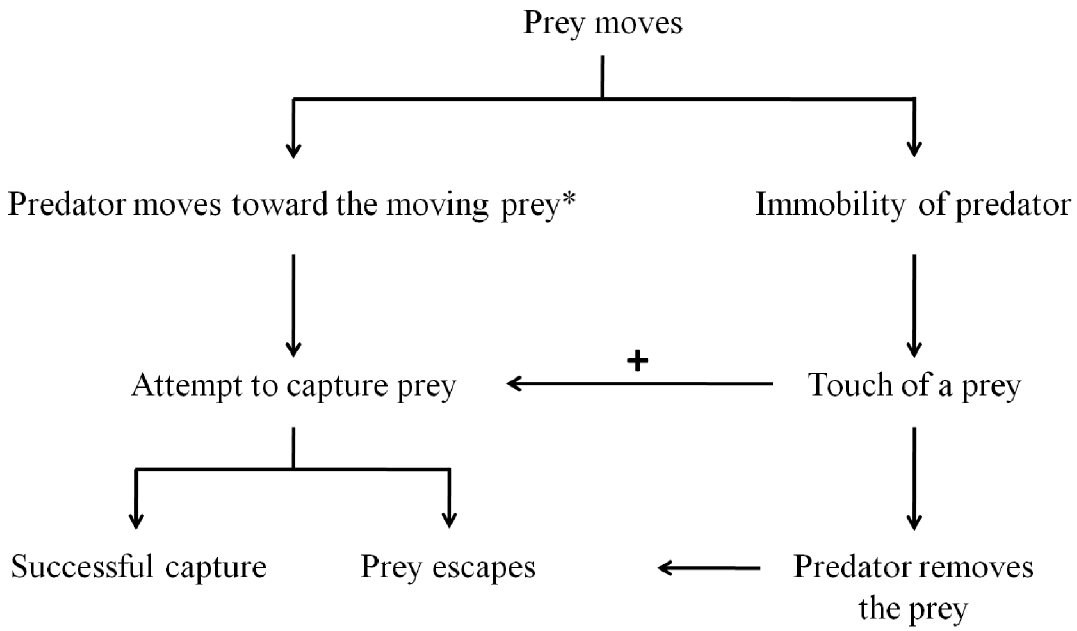

Figure 1. Ethogram based on observed behaviors exhibited by the predators (toads) and its preys (crickets) during the experimental trials. *behavior not observed in blind toads, +behavior not observed in normal toads.

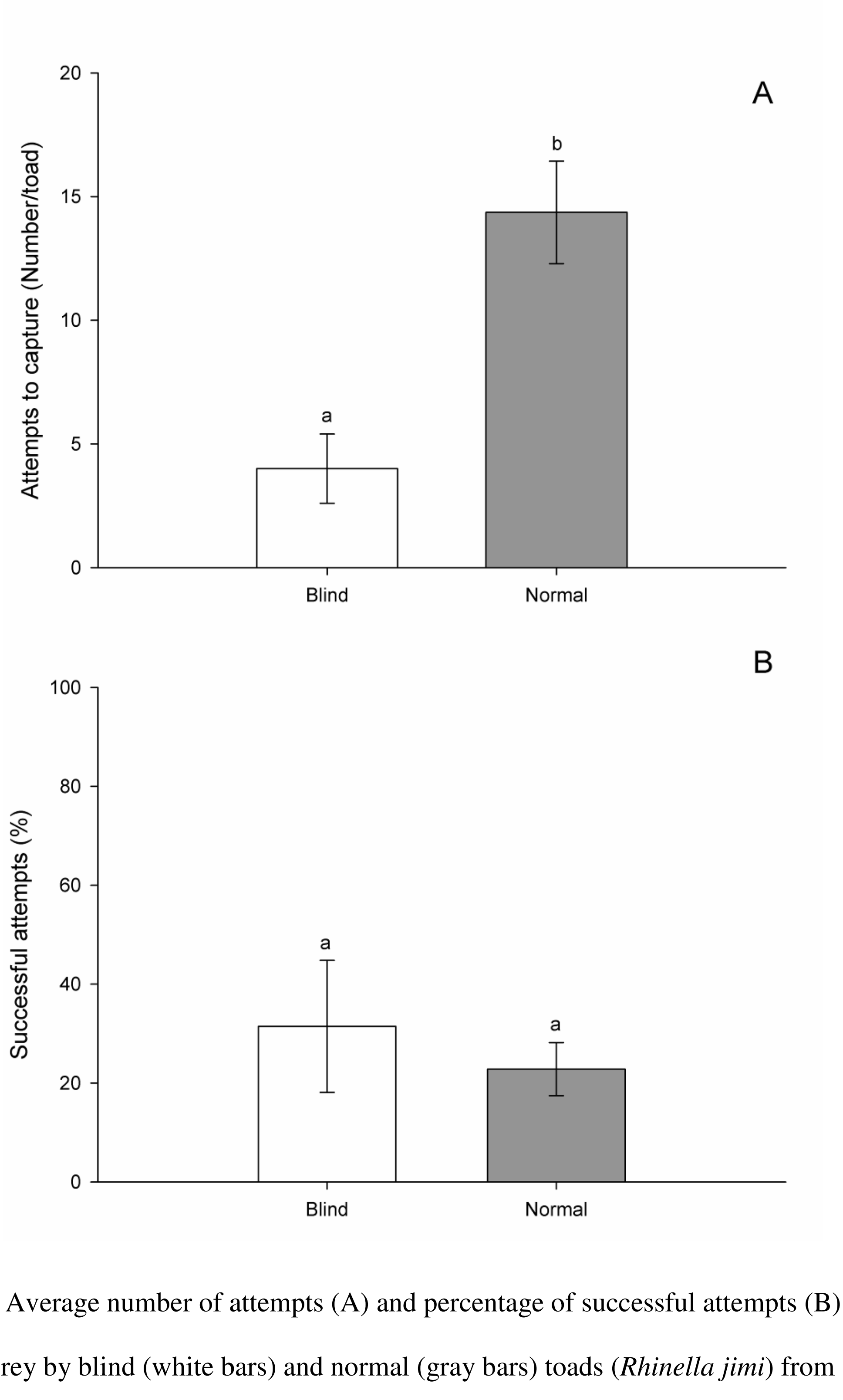

Figure 2. Average number of attempts (A) and percentage of successful attempts (B) to capture prey by blind (white bars) and normal (gray bars) toads (*Rhinella jimi*) from Fernando de Noronha. Different lowercase represent differences between blind and normal toads as indicated by t-test. Means and standard error bars.

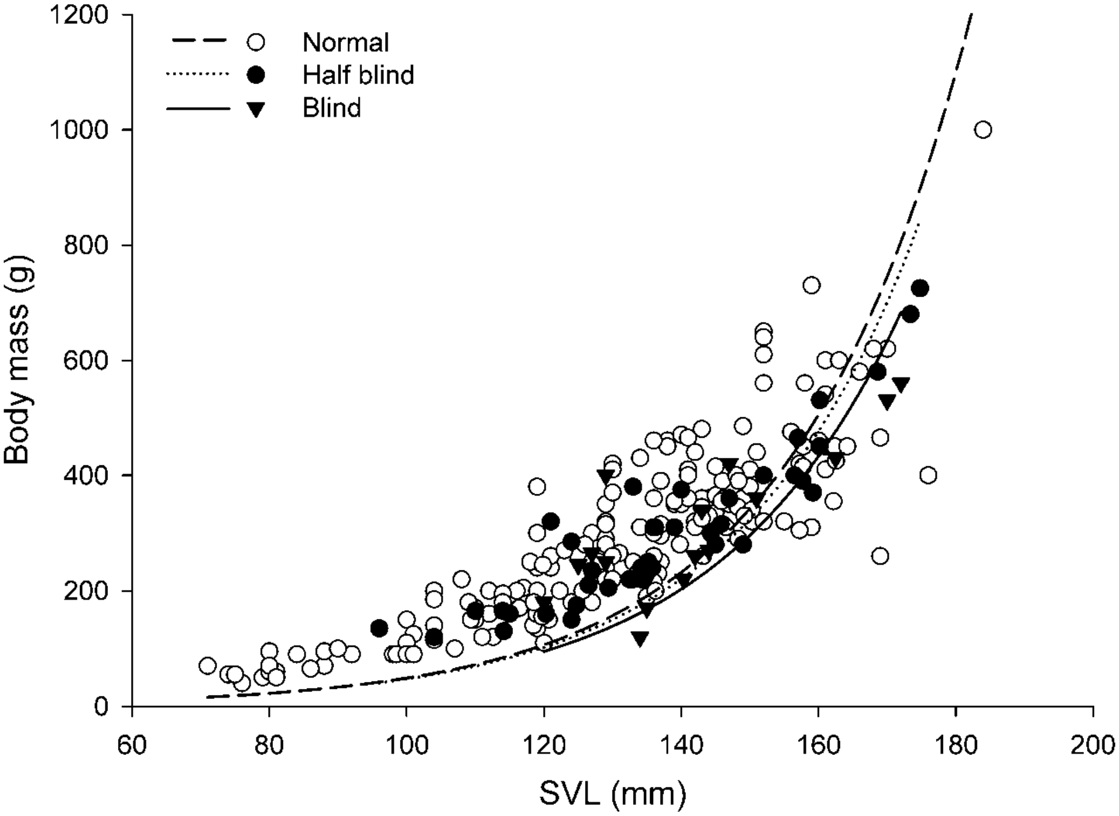

Figure 3. Relationships between body mass and size (snout-vent length) in normal, half blind, and blind individuals of *Rhinella jimi* from Fernando de Noronha.

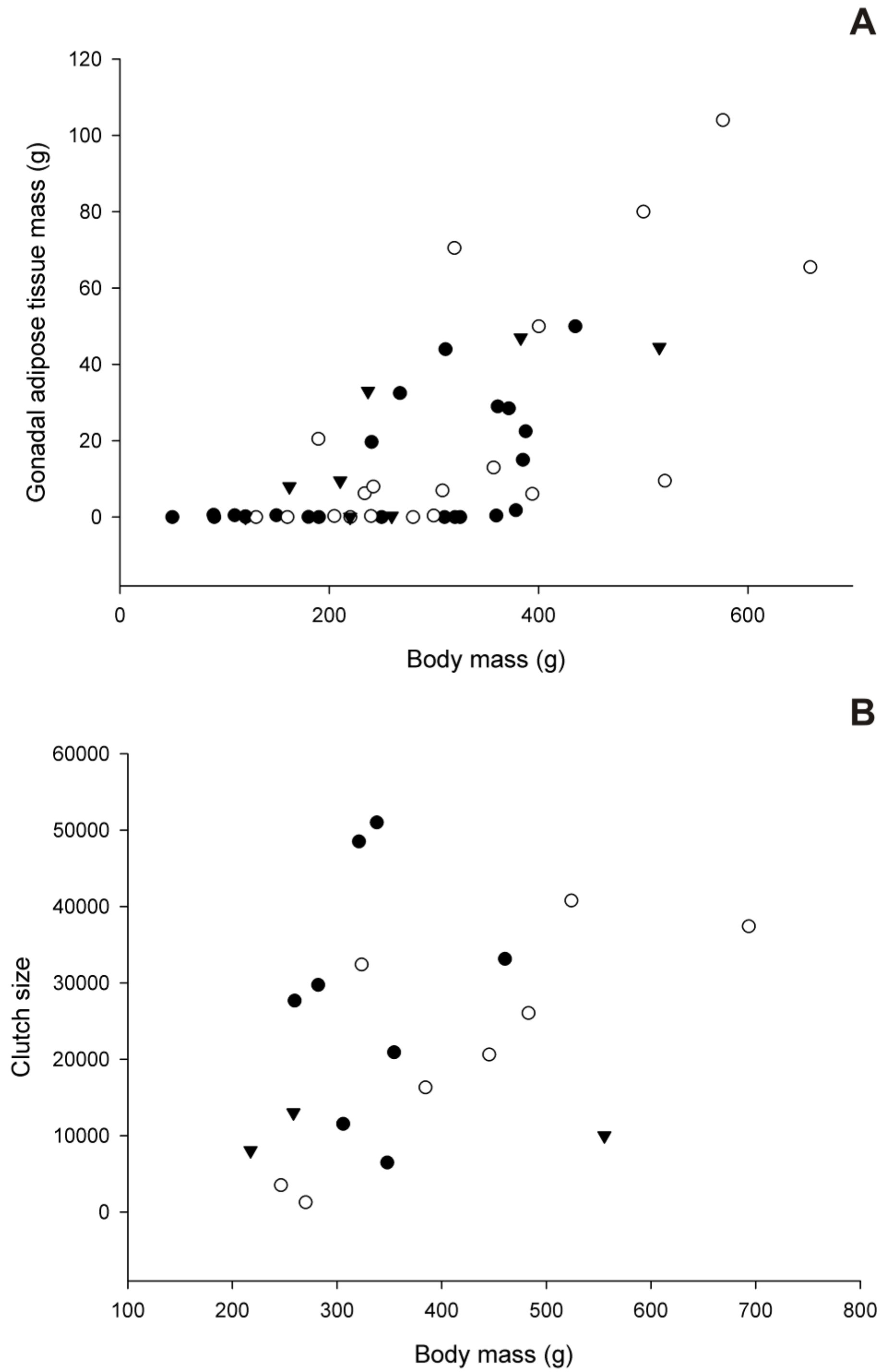

Figure 4. Relationships between body mass and: gonadal adipose tissue (A); and clutch size (B) of normal (closed circles), half blind (open circles), and blind individuals (triangles) of *Rhinella jimi* collected in Fernando de Noronha Island.

CAPÍTULO 5 - Reproduction of *Rhinella jimi*: an introduced species in Fernando de Noronha archipelago

JULIA TOLLEDO & LUÍS FELIPE TOLEDO

JOURNAL OF HERPETOLOGY

LRH: J. Tolledo and L.F. Toledo

RRH: *Rhinella jimi* reproduction

Reproduction of *Rhinella jimi*: an introduced species in Fernando de Noronha archipelago

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Key words: Anuran reproduction, Introduced species, Deformities,

Abstract. *Rhinella jimi* is an introduced species in the archipelago of Fernando de Noronha. The population has a large amount of deformed individuals, including blind toads. In this paper we investigated several aspects of the reproductive biology of this alien population. We registered sexual size dimorphism, with females being larger than males. There was no relationship between female size and its blindness condition with the number of eggs in

their ovaries. The advertisement call of the insular population did not differ of the call of the mainland population. Besides calling, males actively searched for females exhibiting low selectivity, and displayed male displacement behavior.

Most bufonids lay their eggs in lentic water bodies, where the exothrophic tadpoles grow until the metamorphosis (Haddad and Prado, 2005). In species of the genus *Rhinella* spawning are usually large and they are deposited as gelatinous strings into the water. After eclosion, many tadpoles of several species can form schoolings (e.g., Haddad and Sazima, 1992). Clutch size vary a lot between bufonid species (Pombal and Haddad, 2005), and it may depend on female's size and age (Davies and Halliday, 1977; Tejedo, 1992; Duellman and Trueb, 1994; Pombal and Haddad, 2005; Wells, 2007). *Rhinella jimi* is a bunfonid of the *R. marina* species group, closely related to *R. schneideri* (Stevaux, 2002). Advertisement and release calls were recently described with some notes on its reproductive behavior (Garda et al., 2010). This description was based on individuals from the municipality of Natal, state of Rio Grande do Norte, Brazil (Garda et al., 2010), a site included in its original distribution (Stevaux, 2002; Frost 2011).

Rhinella jimi was introduced in Fernando de Noronha probably about 100 years ago (Olson, 1981; Oren, 1984). Since then it established a large population spread all over the main island of the archipelago. Almost half of the tadpole and adult population present external abnormalities (Tolledo & Toledo, unpubl. data; Toledo and Ribeiro 2009). This introduced population breeds virtually in all water bodies of the island, but there is no information about its breeding biology in the island, especially in relation of the possible influences of their malformations. About 20 % of the deformed individuals present eye deformities, with some being completely blind (Toledo and Ribeiro, 2009). Blind individuals feed less frequently than normal ones, leading to a lighter body mass condition (Tolledo et al., unpubl. data). This reduced efficacy in foraging and growing, associated to the possible difficulty in locating females (in blindness condition), can led blind individuals to be in disadvantage or to make use of different reproductive strategies in relation to normal males.

Therefore, we investigated some aspects of the reproductive biology of the alien population of *R. jimi*. We described the advertisement call of the insular population and compared it to the mainland population (Garda et al., 2010). We did observations on their reproductive behavior by means of mark and recapture survey. Finally, we calculated the size relationships between males and females, as well as relationships about female reproductive effort.

Material and Methods

Study site

Fernando de Noronha is an oceanic archipelago situated in northeastern Brazil, where all available freshwater bodies (ponds and rivulets) came from rain. Field work was done in the margins of these water bodies, mainly in two of them: Açude da Quixaba and Açude da Ema. Açude da Quixaba (38W 26' 16", 3S 51' 28") is a small artificial pond in a private property. The pond is shallow, with maximum deep about 50 cm. There were large amounts of the macrophyte *Salvinia* sp. inside and in the edge of the pond. Vegetation in the edge was composed by herbaceous plants and there was a small bean farm near the pond. Açude da Ema (38W 25' 34", 3S 50' 59") is the second largest water reservoir of the island with 11,280 m^2 of surface area. There were small amounts of herbaceous vegetation inside the water and the marginal vegetation was composed mainly by bushes.

Field sampling

In April and May 2010 we collected adults of *R. jimi*, determined their sex based on secondary characters (i.e., presence of nuptial pads and vocal sac in males; both absent in females), and measured their snout-vent length (SVL) in order to investigate differences between sexes. Some females were killed, had their ovaries removed, and we then counted the total number of oocites to determine relationships between body size and number of eggs produced (as a measure of reproductive effort).

Between 4th and 23rd May we made 10 field activities to observe the reproductive behavior of *R. jimi* in Açude da Ema. We captured and marked all adults we found, and the recaptures were registered. We registered how many males were calling, how many individuals were in the pond each night, and their location was georreferenced. We also measured the distance between males in calling activity. The marking method used was toe clipping (Donnelly et al., 1994) and the numbers were based on Martof (1953).

Call description

We recorded from one to four advertisement calls of five toads in Açude da Quixaba in May 2010. These toads were measured for their SVL and body mass. Air temperature was registered after each recording. We used a Marantz cassette tape recorder (PMD222) equipped with an external directional microphone (Audiotecnica AT835b) positioned approximately 2 m from the calling males. Calls were digitalized in 48 kHz at 16 bits of sampling size and saved in .wav format. FFT and frame length of 256, brightness 80 % and contrast 85 %. We analyzed call parameters in Raven Pro 1.3 (Charif et al., 2008) and the terminology follows Toledo and Haddad (2005).

Data analysis

Sexual size (SVL) dimorphism was analyzed with a t-test. The relationships between female body mass and SVL with the number of eggs produced were assigned by means of linear regression analysis, being the liquid body mass considered as the total body mass subtracting the ovaries mass. As there were no effects of body mass or SVL in the number of eggs produced, the comparison between blind, half blind and normal females in the amount of eggs produced was done by means of an analysis of variance (ANOVA). Reproductive efforts were calculated as the ratio of clutch to body mass (Gadgil and Bossert, 1970), and were compared by means of an analysis of variance (ANOVA). To compare distances of males and females from the pond we did a t-test. For the individuals captured more than once, we did an average of distance from the pond in all encounters. Before all analysis we did Levene's tests to homogeneity of variance and we visually analyzed normality. When necessary we transformed data according to recommendations of Zar (1999).

Results

Snout-vent length was measured for 134 males and 81 females. Females (SVL = 144.80 ±17.21 mm; range: 110 to 184 mm) are larger than males (SVL = 133.15 ±15.71 mm; range: 96 to 173 mm) ($t_{1,213} = 5.08$, $P < 0.001$; Fig. 1). The mean number of oocites produced per female was 25,271 ± 12,744 (range: 3,540 to 51,012; N = 27). Number of oocites produced was not correlated to female liquid body mass ($R^2 = 0.13$; $P = 0.07$; N = 19; Fig. 2), neither to SVL ($R^2 = -0.04$; $P = 0.56$; N = 19). The number of eggs per female and the reproductive effort did not differ between blind, half blind and normal individuals ($F_{2,25} = 2.99$; $P = 0.068$; and $F_{2,18} = 2.46$; $P = 0.117$; Fig. 3).

We marked a total of 38 individuals (11 females and 27 males). One of the females was recaptured once and the others were not recaptured. Among males, 12 were recaptured at least once, and up to five times (one male), and the others 15 males were not recaptured. There were no difference between males and females on the distance from the pond ($t_{1,36} = 0.93$; $P = 0.35$).

Besides the calling male strategy, we also observed alternative sexual strategies, such as active searching for females in 3 out of 30 nights. This behavior was observed in nights that there was a large amount of toads in the pond. While searching (swimming in the pond or jumping around its margins) for females these males amplected all sorts of things they encounter, including stones, aquatic plants, fallen trunks, dead toads (Fig. 4), plastic bottles, and the boots of the observer. Besides this, we also registered an attempt of male displacement by other male. In this situation the female stayed all the time under the water and the displacement male was not successful and went away after the interaction. We observed blind males in active searching for females and one blind individual calling. We also observed a blind female in amplexus with a normal male and a couple of blind toads in amplexus.

We analyzed ten calls of five individuals of *R. jimi* from Fernando de Noronha. The advertisement call was multipulsed, without harmonics, and varied in duration from 4.9 to 14.1 seconds (average of 7.54 ± 2.61 s) and in dominant frequency from 375 to 562.5 Hz (average of 487.97 ± 80.47 Hz) (Fig. 5). Notes presented 2 pulses and the average number of notes per call was 110.9 ± 24.11. The initial portion of all calls had an ascendant structure composed by 3 to 20 notes (average of 9.9 ± 5.61). Most of the calls ended abruptly, but two of them presented a final descendant portion composed by 9 and 12 notes. Four calls had a little gap varying from 0.07 to 0.17 seconds. Air temperature during the call records was between 25.7 and 27.7 ºC.

Discussion

Females are larger than conspecific males in almost 90 % of anuran species (Shine, 1979). This may be a consequence of age, where females achieve older ages than males

(Monnet and Cherry, 2002), or could be a result of selective pressures larger in females than in males (Arak, 1988). *Rhinella jimi* was referred to present the opposite pattern, with males (147.48 ± 16.34 mm) larger than females (133.8 ± 29.71 mm) in its taxonomic description (Stevaux, 2002). However, our data demonstrates that females are larger than males in Fernando de Noronha. This observation could be explained by the smaller sampling size of the former study [N = 60 in Stevaux (2002) against N = 215 by the present study], or it is possible that there are morphological differences between the insular and continental populations.

The number of eggs produced per female *Rhinella jimi* (in average more than 25,000) was larger than that reported for other bufonid species, like *Anaxyrus houstonensis* with a maximum clutch size of 6,200 eggs (Quinn and Mengden, 1984), *A. cognatus* with an average of more than 11,000 eggs (Krupa, 1994), *R. ornata* with an average of almost 4,700 eggs, *R. icterica* with an average of almost 19,700 eggs (Pombal and Haddad, 2005), and *R. marina* with an average varying from 10,000 to 32,000 (Cohen and Alford, 1993). Species of the *R. marina* group are probably those that lay the highest number of eggs among all living anurans.

Large clutch size is related to a wider distribution area (van Bocxlaer, 2010), because of the capability to colonize a wider range of environments. It can be also linked to r-strategy (*sensu* MacArthur & Wilson, 1967), with adults producing a large amount of offspring and with rapid development (Pianka, 1970). *Rhinella jimi* presents traits related to success on invasion and colonization of new areas, such as a diet widely variable (Tolledo, Ribeiro & Toledo, unpubl. data), high tolerance to environmental conditions, and short developmental time. Moreover, the skin resistant to desiccation (Duellman and Trueb, 1994) allows *R. jimi* to live in xeric environments, such as sandy beaches in Fernando de

Noronha. Along with the absence of natural predators (such as snakes, large mammals, and nocturnal birds) in the Island, we suggest that the predation pressure upon *Rhinella jimi* in Fernando de Noronha is reduced when in comparison to the predation pressure of the inland population. All these factors may contribute to the successful invasion of the Island by *Rhinella jimi*.

Body mass and SVL were not related to number of eggs in *Rhinella jimi* as it was observed to other Bufonidae species (e.g., *Bufo bufo* Davies and Halliday, 1977; *Epidalea calamita* Tejedo, 1992; *Pseudepidalea viridis* Castellano et al., 2004, Baskale et al., 2011), but there is a possible sample size bias as the *P* value was 0.07 and it is possible to observe a trend in increasing the number of eggs with the body size. Blindness did not affect number of eggs produced, however this result was also marginally significant ($P = 0.068$) and we suggest there may be an effect but we could not detect it, once more, due to reduced sample size (N = 3 blind gravid females).

We observed behaviors attributed for both reproductive strategies proposed by Wells (1977) - explosive and prolonged breeders - in toads from Fernando de Noronha, such as males calling from calling sites and males in active search for females. Active searching for females is pointed out as a trait of explosive breeding species (Wells, 1977), although it can also occur in prolonged breeding species when the male density is elevated (Wells, 2007). We suggest that *Rhinella jimi* in Fernando de Noronha is a prolonged breeder (*sensu* Wells, 1977), since there was calling activity for more than a month during the local wet season. Also, we observed breeding activity and tadpoles almost exclusively in the wet season (with one register of breeding activity and one of tadpoles during 30 days of field work in the dry season), suggesting this population breed mainly in the wet season.

Most of the call parameters described here are not different from that described previously for the mainland population (Garda et al., 2010). But individuals from Fernando de Noronha presented lower dominant frequency, ranging between 375 and 562.5 Hz while mainland individuals varied between 516.8 and 689.1 Hz (Garda et al., 2010). This difference may be a result of differences in male size (between 147.3 and 152 mm in the Island and 105.97; and 114.02 mm in mainland), since dominant frequency and male size are inversely correlated to some species (e.g., *Anaxyrus woodhousii* Sullivan, 1989).

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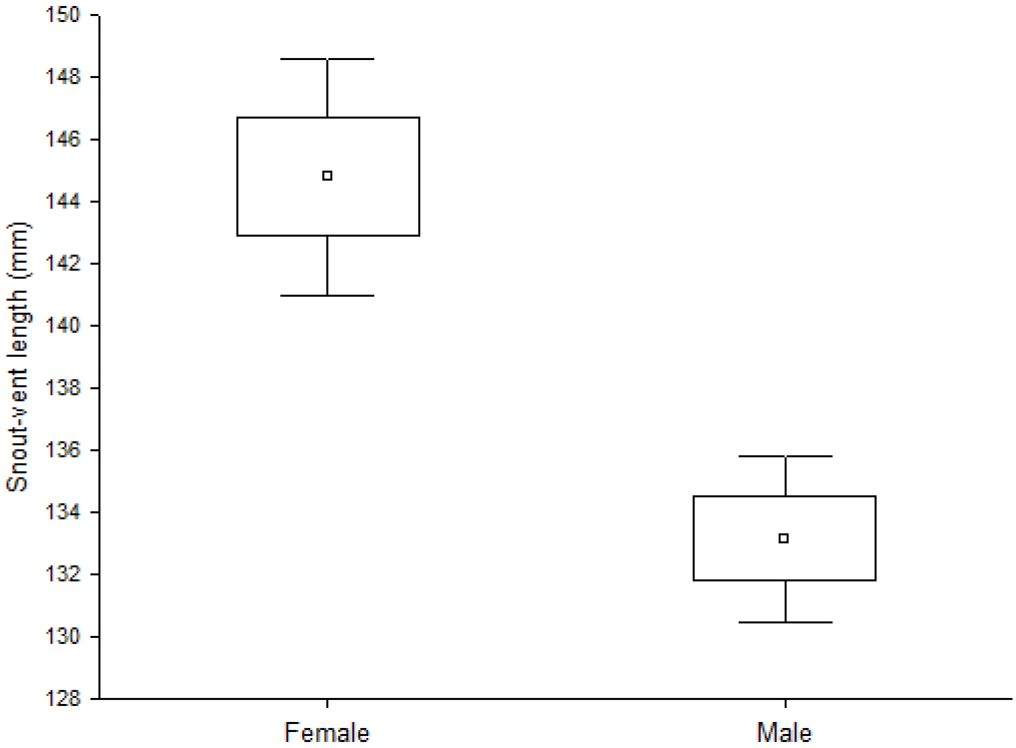

Figure 1. Snout-vent length of females (n = 81) and males of (n = 134) *Rhinella jimi* from Fernando de Noronha. The points represent mean, the boxes are ± standard error, and the bars are ± confidence interval to 95 %.

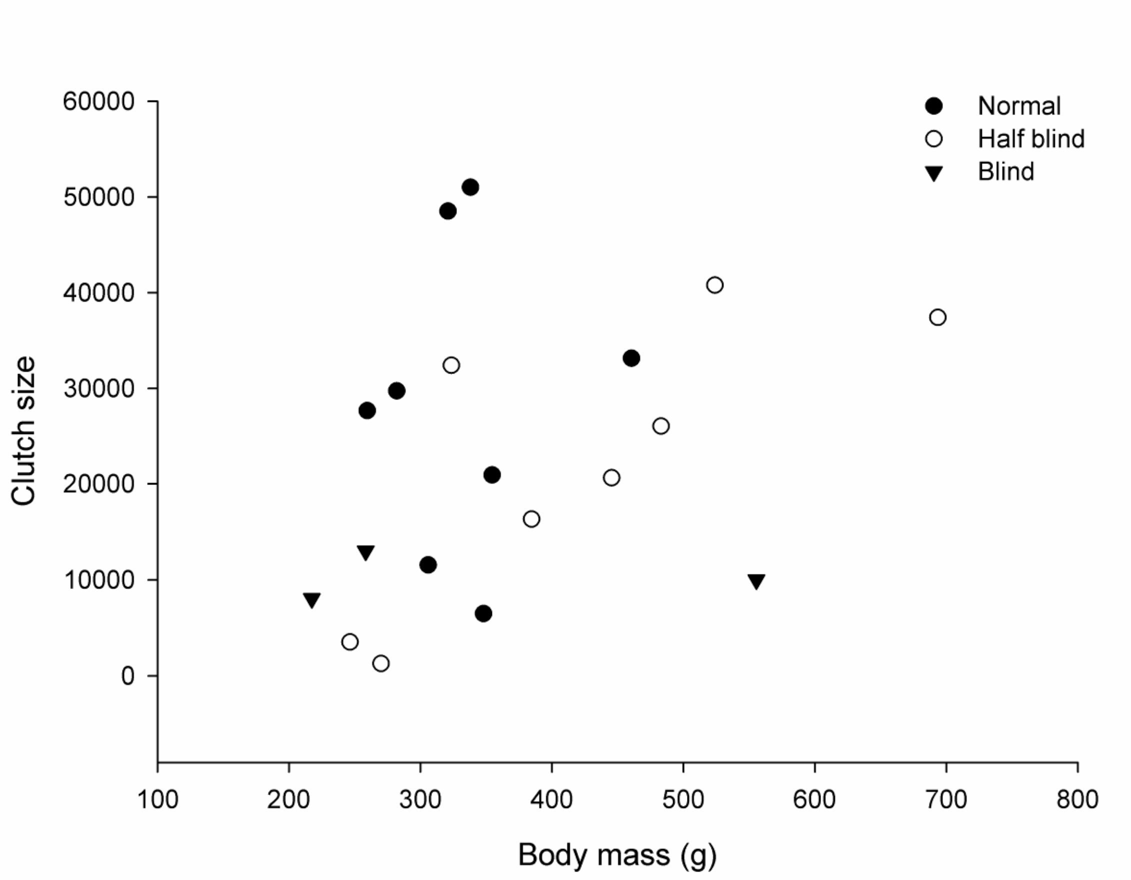

Figure 2. Clutch size in relation to liquid body mass in normal, half blind, and blind females of *Rhinella jimi* from Fernando de Noronha.

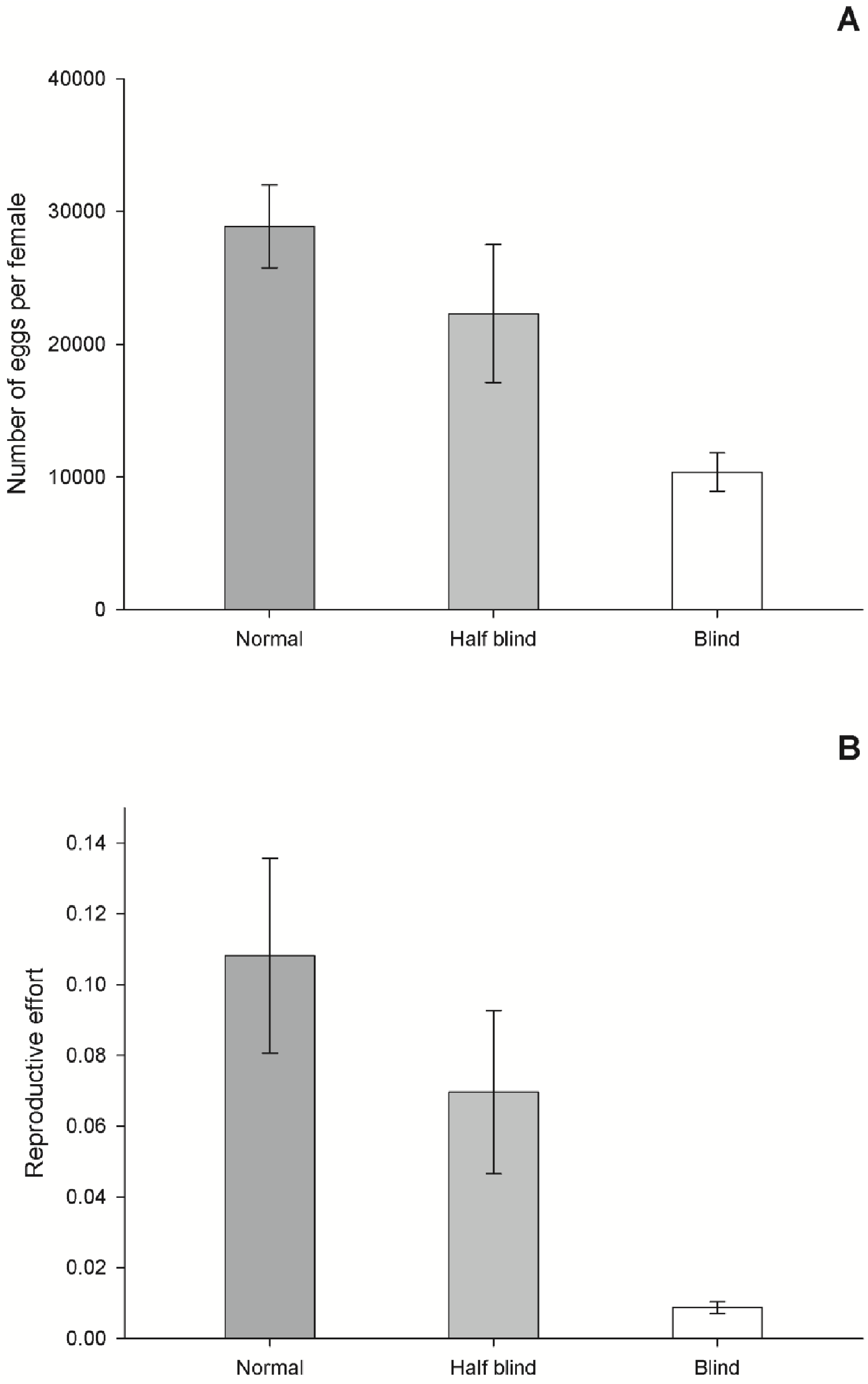

Figure 3. Number of eggs (A) and reproductive effort (B) per normal, half blind, and blind females of *Rhinella jimi* from Fernando de Noronha. Bars are standard errors.

Figure 4. Adult male *Rhinella jimi* in amplexus with a dead conspecific female after displaying the active searching strategy.

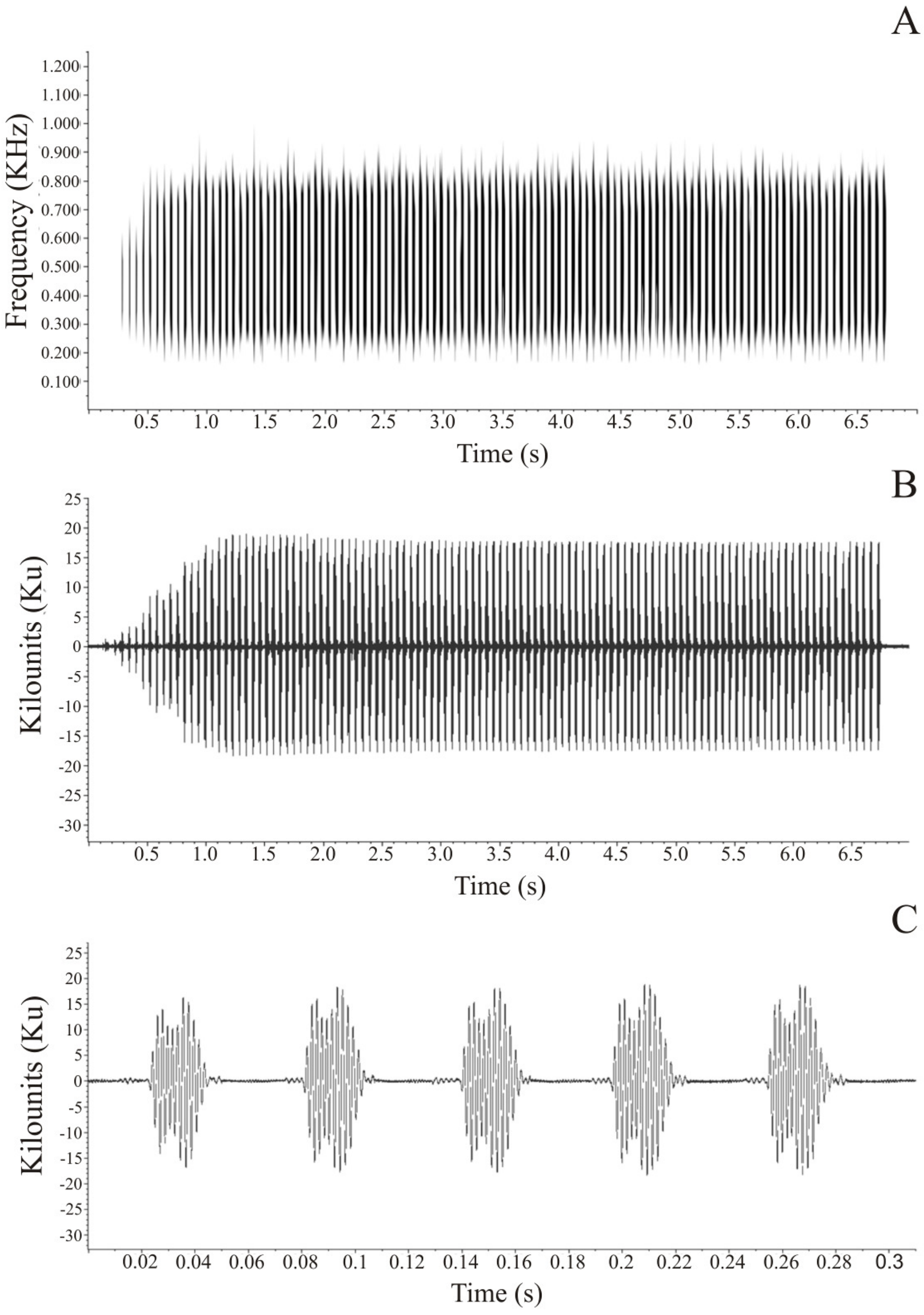

Figure 5. Advertisement call of male *Rhinella jimi* from Fernando de Noronha. Waveform of a call (A); spectrogram of the call presented in A (B); and spectrogram of five notes showing two peaks per note (C). Air temperature 26.4 ºC.

Conclusões gerais

Com este trabalho foram colhidas informações relevantes sobre uma população peculiar de anuros introduzida em uma ilha oceânica. O sistema de estudo é de grande interesse para estudos evolutivos bem como para o entendimento dos efeitos de deformidades em populações naturais. As principais conclusões a que pudemos chegar estão citadas abaixo:

- Assim como os adultos, girinos da população de *Rhinella jimi* de Fernando de Noronha apresentam alta taxa de deformidades (mais de 50%).
    - Estas deformidades estão associadas a desvantagens relacionadas à alimentação
- Existem diferenças entre a dieta de indivíduos caolhos e normais de *Rhinella jimi* da população de Fernando de Noronha
- Sapos cegos desta mesma população usam o tato para localizar e capturar presas
    - Ser cego parece trazer algumas desvantagens para estes indivíduos, como uma menos na massa corpórea
- Existe dimorfismo sexual de tamanho, com fêmeas maiores que os machos na população de *Rhinella jimi* de Fernando de Noronha
- A vocalização dos machos de *Rhinella* jimi de Fernando de Noronha é semelhante à de indivíduos do continente (coletados em Natal)
- Indivíduos de *Rhinella jimi* cegos parecem se reproduzir, visto que observamos a presença de casais em amplexo

Considerações finais

O trabalho respondeu algumas questões relevantes sobre a história natural da população estudada e levantou outras questões que podem ser respondidas em trabalhos futuros. Sendo assim, deixamos sugestões de perguntas a serem investigadas em outros trabalhos que envolvam a mesma população. São elas:

1) A salinidade afeta o desenvolvimento de girinos?
2) A radiação UV-B pode causar malformações em girinos de *R. jimi*?
3) Temperaturas elevadas podem causar malformações em *R. jimi*?
4) Girinos deformados dão origem a adultos deformados?
5) A prole de um casal deformado será composta por indivíduos deformados?
6) Existe contaminação na água de Fernando de Noronha? Isto causa malformações?
7) A causa da malformação é genética? Causada por endogamia e efeito de gargalo, já que esta é uma espécie introduzida?
8) A população introduzida está causando impactos negativos sobre a fauna nativa de Fernando de Noronha?
9) Qual o tamanho e densidade populacional de *R. jimi* em Fernando de Noronha?
10) As malformações são causadas por parasitas ou doenças?

As questões acima foram levantadas ao decorrer do presente estudo e alguns dos itens já estão sendo pesquisados em estudos paralelos; os quais devem contribuir significativamente para conservação do arquipélago de Fernando de Noronha.