



Anomalous tadpoles in a Brazilian oceanic archipelago: implications of oral anomalies on foraging behaviour, food intake and metamorphosis

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Rhinella jimi (Anura, Bufonidae) is an introduced species in the archipelago of Fernando de Noronha, north-eastern Brazil. It is known as one of the greatest amphibian anomaly hotspots in the world, with almost half of the adult individuals in the population having external anomalies, but tadpoles from this population have not previously been examined. Therefore, we evaluated the presence of anomalies in tadpoles of this population, described their types and identified possible handicaps of anomalous tadpoles in foraging behaviour and food intake. We found anomalies in 52.5% of all tadpoles inspected, mostly involving labial teeth. Anomalous tadpoles, when compared to normal individuals, spend less time foraging and have a lower foraging efficiency. We also observed that anomalous toadlets originate both from normal and anomalous tadpoles. We suggest that the reduced feeding fitness may result in a reduced growing rate, longer time spent until metamorphosis, higher predation risk, different body mass, size and morphology in metamorphs and adults. However, this apparent handicap may not affect the post-metamorphic population, as anomalous adults may rise from normal tadpoles.

Key words: amphibian anomalies, feeding, handicapped individuals, introduced species

INTRODUCTION

Amphibians are declining worldwide due to multiple causes (Blaustein et al., 2011) and the prevalence of anomalous individuals, a possible factor contributing to amphibian declines, may be increasing in recent years (Lannoo, 2008). Anomalies caused by mutations, developmental errors and trauma are common, but they generally occur in 5% or less of the individuals in a population (Blaustein & Johnson, 2003; Lunde & Johnson, 2012). However, recent observations reported higher rates of anomalies (15–90%) caused by many different factors (Blaustein & Johnson, 2003). Most of the available information in amphibians focuses on post-metamorphic individuals documenting mainly limb and digit anomalies (Ouellet, 2000; Toledo & Ribeiro, 2009; Lunde & Johnson, 2012). Studies on oral anomalies of larval anurans are scarce, but have demonstrated high rates of anomalies (6–98%) either in field sampled populations (Burger & Snodgrass, 2000; Drake et al., 2007; Bacon et al., 2013) or based on tadpoles deposited in museum collections (Medina et al., 2013). Tadpole oral anomalies can be caused by many factors, for example seasonal changes (Rachowicz, 2002), pollution (Rowe et al., 1998; Bacon et al., 2013), temperature (Bresler, 1954) and diseases

such as chytridiomycosis (Fellers et al., 2001; Drake et al., 2007; Vieira et al., 2013). Anomalies in oral parts may affect tadpole feeding behaviour, the total amount of food intake and growth (Rowe et al., 1996; Venesky et al., 2009, 2010).

In the Brazilian oceanic archipelago of Fernando de Noronha, there is a high rate (almost 45% of the population) of anomalous adult individuals of the introduced population of *Rhinella jimi* (Anura; Bufonidae) (Toledo & Ribeiro, 2009). This rate is greater than that observed in the natural range of the species, which varies from about 4–10%. The causes of this high occurrence of anomalous toads are yet unknown and some suggestions have been made (Toledo & Ribeiro, 2009). Toledo & Ribeiro (2009) examined only post-metamorphic individuals (mainly adults). Therefore it was not possible to determine if anomalies 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 anomalies in tadpoles of the same population. Furthermore, if anomalies were observed, we evaluated if they would affect the behaviour and development of tadpoles.

Jaw sheaths and keratinised labial teeth are directly involved in the acquisition of food, responsible for grazing and for adherence on substrate (Wassersug & Yamashita, 2001; Venesky et al., 2010, 2013). Therefore, we expect tadpoles with oral anomalies must spend less time in the substrate and must acquire less food than normal tadpoles.

MATERIALS 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, on the main island of Fernando de Noronha, state of Pernambuco, Brazil (3°50'S, 32°25'W; sea level). Additional collecting was made in April 2011 for the metamorphosis experiment. Specimens analysed for anomalies were fixed in 7% formalin solution and deposited 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, 16688, 18614–17).

Anomaly evaluation

We used the term "anomaly" to describe all abnormal morphology observed, as suggested by Altig (2007) when its causes are unknown. All collected tadpoles were inspected with a stereomicroscope and assigned to a larval stage (Gosner, 1960). Tadpoles were compared with those described by Tolledo & Toledo (2010). Oral anomalies were classified according to Drake et al. (2007) except by the marginal papillae, which were not examined. Oral anomalies were assigned to seven regions of the oral apparatus (Fig. 1). Anomalies not described by Drake et al. (2007) or by Medina et al. (2013) were here characterised.

Experiments

To test the influence of oral anomalies in foraging behaviour and efficiency we performed two experiments, similar to those reported by Venesky et al. (2009).

In experiment 1 we compared foraging behaviour of normal and anomalous tadpoles measuring the time spent on foraging activity. Tadpoles were assigned to three developmental stages: early (Gosner 26–29); intermediate (Gosner 30–34); and late (Gosner 35–40) ($n=10$ for each stage, but $n=12$ for anomalous tadpoles in the early stage). Tadpoles were placed in 15x10 cm plastic containers, with 3 cm deep (450 ml) clean water and a stone encrusted with algae (which was abundant on the stone so that the tadpole was not limited in food availability). These stones were collected in the field after observations of natural feeding. After an acclimation period of two minutes, an experimenter (who did not know if the tadpole was anomalous 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.

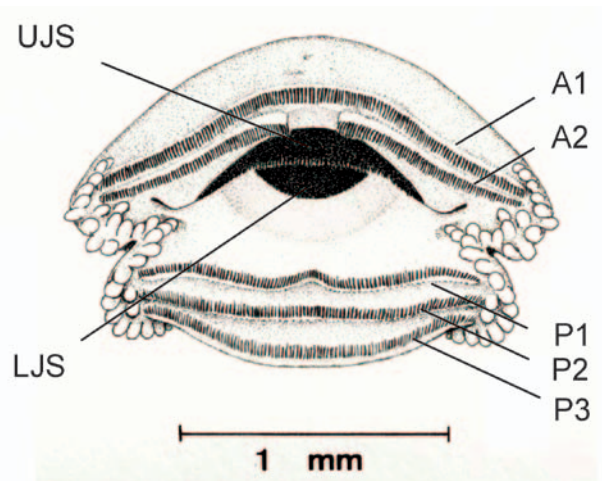


Fig. 1. Oral apparatus of a normal *Rhinella jimi* tadpole, indicating the seven regions evaluated for anomalies: A1–2: anterior tooth rows 1 and 2; P1–3: posterior tooth rows 1, 2 and 3; UJS: upper jaw sheath; LJS: lower jaw sheath. Modified from Tolledo & Toledo (2010).

In experiment 2 we tested whether oral anomalies negatively affect tadpole foraging efficiency, examining the quantity of food consumed during one 3-hour trial. The tadpoles were divided as in experiment 1 (normal vs. anomalous), $n=10$ for each stage, but $n=9$ and $n=8$ for normal tadpoles in middle and late stages, respectively. Tadpoles were kept in 4x5 cm diameter plastic containers (two tadpoles per container) with 40 ml of clean water and were starved for three days. The water was changed regularly to avoid ingestion of faeces. Tadpoles were then placed in 700 ml plastic containers (ten tadpoles per treatment) with algae covered stones (as in experiment 1).

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

A third experiment was implemented in order to evaluate the assumption that abnormal tadpoles metamorphose into abnormal toadlets, and normal tadpoles metamorphose into normal toadlets. Therefore, we maintained 167 tadpoles (67 normal and 100 anomalous, initially in Gosner stage 25) in the laboratory (air temperature 25°C, food *ad libitum*), and kept them until they died or completed metamorphosis. After completing metamorphosis they were killed and fixed for morphological examination. The tadpoles were kept in six plastic trays (three trays for the normal and three for the anomalous ones), each one with about 2 litres of potable water, which was changed daily. The plastic trays measured 50x100 cm with around 30 tadpoles in each tray, (varying from 23 to 34) resembling similar densities when the tadpoles swim in schools in natural conditions (LFT, pers. obs.).

Table 1. Types of anomalies, number and percentage of anomalous *Rhinella jimi* tadpoles (out of 413 individuals sampled) from the Fernando de Noronha archipelago, Brazil. *indicates anomalies not described by ¹Drake et al. (2007) or ²Medina et al. (2013).

	Code	Type of anomaly	Affected tadpoles
Jaw sheath (JS)	JS1	Breaks, gaps, or other deformities in the cutting edge ¹	10 (2.42%)
	JS2	Breaks, gaps, or other deformities in the base of the jaw sheath ¹	01 (0.24%)
	JS3	Lack of keratinization in the jaw sheath ¹	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 middle of the superior jaw sheath*	01 (0.24%)
Tooth rows (TR)	TR1	Missing teeth ¹	18 (4.36%)
	TR2	Missing teeth with disrupted supporting tissue ¹	171 (41.40%)
	TR3	Duplication of teeth (e.g., double row, circular arrangement) ¹	09 (2.18%)
	TR4	Stunted teeth ¹	91 (22.03%)
	TR5	Intersecting tooth rows ¹	55 (13.32%)
	TR6	Puckering (sharp convolutions) of tooth rows ¹	16 (3.87%)
	TR7	Overlapping tooth rows (division of the tooth row and the overlapping of the two resulting rows) ²	16 (3.87%)
	TR8	Missing tooth row*	81 (19.61%)
	TR9	Tooth row directed antero-posteriorly (teeth in a 90° rotated position in relation to normal ones)*	16 (3.87%)
	TR10	Short tooth row*	28 (6.78%)
	TR11	Completely lost of anterior labia*	4 (0.97%)
	TR12	Completely lost of posterior labia*	2 (0.48%)
	TR13	Abnormal shape in tooth rows (other than the cited)*	10 (2.42%)

Data Analyses

Linear regression analysis was utilised to compare prevalence of anomalies with developmental stage, using developmental stage as the independent variable and the arcsine transformed data of proportion of anomalous tadpoles as the dependent variable (Zar, 1999). We used a chi-square analysis to compare anomaly prevalence between larvae and adults, using data from Toledo & Ribeiro (2009) for adults and data from this study for tadpoles.

We applied a two-way ANOVA to evaluate the impact of the presence of oral anomalies and developmental stage on time spent foraging and on the efficiency of food intake. Anomaly and developmental stage were the factors in both tests, and the dependent variables were the arcsine square-root transformed data of proportion of time spent on foraging and proportion of full gut, respectively. When the null hypothesis was rejected, a Tukey *post-hoc* test was applied (Zar, 1999).

RESULTS

A total of 413 tadpoles between stages 26 and 40 (Gosner, 1960) were analysed, from which 217 (52.5%) exhibited anomalies to some degree. All abnormal tadpoles presented oral anomalies, two had nostril anomalies and ten had anomalous toes. A total of 997 oral anomalies was observed, being 964 (96.7%) in tooth rows and 33 (3.3%) in jaw sheaths (Table 1). Among the 964 anomalies observed in tooth rows, 47% (453) were in anterior tooth rows, 291 at “A1” and 162 at “A2”; and 53% (511) were in posterior tooth rows, 98 at “P1”, 194 at “P2”, and 219 at “P3”. Twenty-seven anomalies were observed in the upper jaw sheath and six in the lower jaw sheath. Oral anomalies were of 19 types, of which nine have already been reported by Drake et al. (2007), one was reported by Medina et al. (2013) and nine were novel and described here (Table 1; Fig. 2A–L). Among the 72 tadpoles with toes fully differentiated (Altig & McDiarmid, 1999) (between stages 37 and 40) ten (13.9%) presented reduced toes (Fig. 2K). One tadpole did not have the left nostril (Fig.

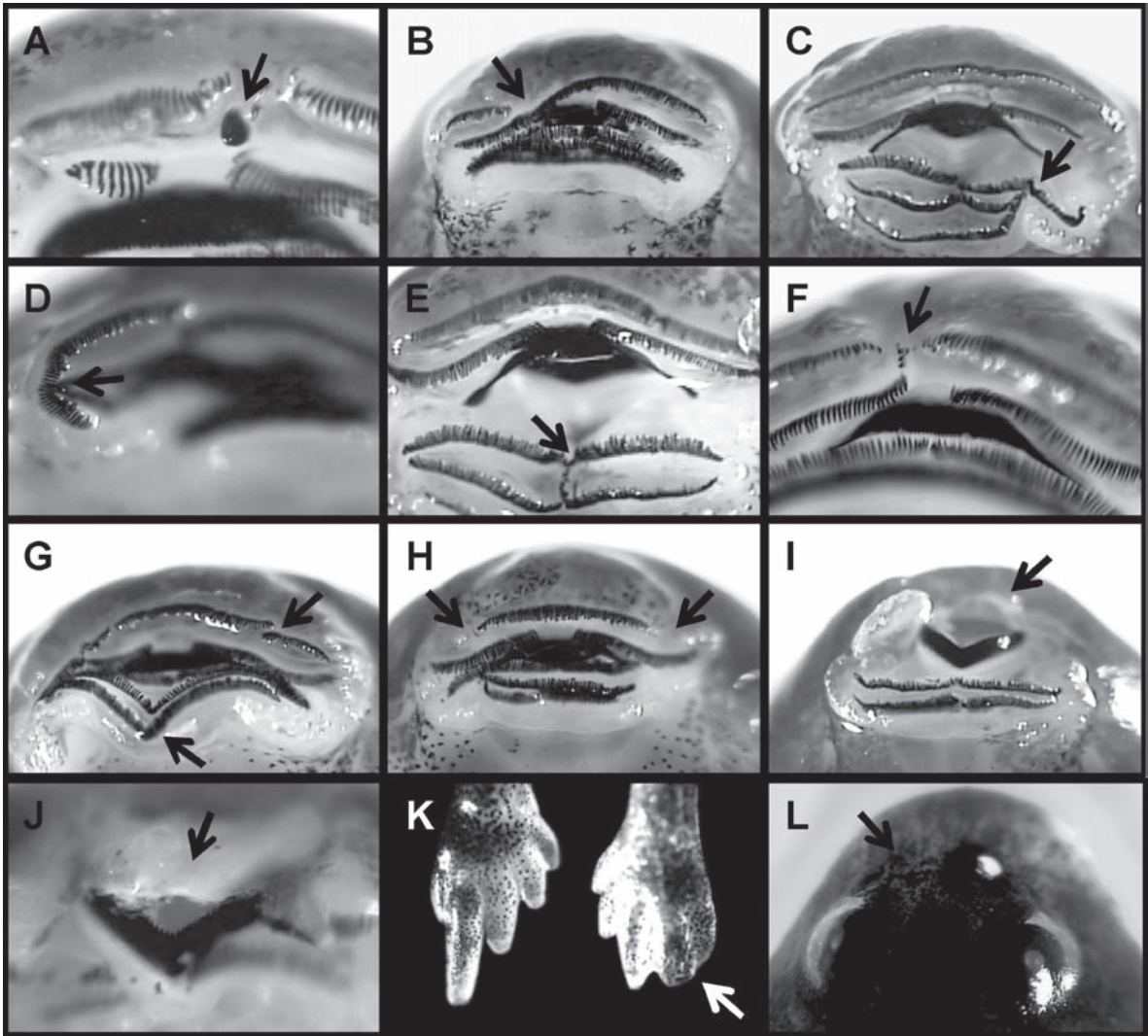


Fig. 2. Anomalies registered in *Rhinella jimi* tadpoles of Fernando de Noronha. (A) Missing teeth with disrupted supporting tissue (TR2) with an abnormal keratinised structure in A1; (B) Intersecting tooth rows in A1–2 (TR5); (C) Intersecting tooth rows in P1–2–3 (TR5); (D) Puckering of tooth row in A1 (TR6); (E) Intersecting tooth rows and tooth row directed antero-posteriorly in P1–2 (TR5–9); (F) Tooth row directed antero-posteriorly in A1 (TR9); (G) Overlapping tooth row in A1 (TR7) and abnormal shape in tooth rows P2–3 (TR13); (H) Short tooth row in A1 (TR10); (I) Completely lost of anterior labia (TR11) and Lack of upper jaw sheath (JS4); (J) Lack of keratinisation in the jaw sheath in UJS (JS3); (K) left foot with shorter toe IV; (L) absence of the left nostril. Codes are in agreement with Table 1.

2L) and another tadpole had a shortened right nostril. The proportion of anomalous tadpoles were not related to its developmental stage ($r^2=0.17$; $F_{1,13}=3.85$; $p=0.07$; Fig. 3). There were no differences between the amount of anomalous adults and tadpoles ($\chi^2=2.86$; $p=0.09$).

The foraging behaviour experiment showed that oral anomalies ($F_{1,56}=4.06$, $p=0.05$), 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. 4). The foraging efficiency experiment revealed a negative effect due to oral anomalies ($F_{1,51}=26.41$, $p<0.001$), but no effects of developmental stage ($F_{2,51}=0.13$, $p=0.88$) or of the interaction between these factors ($F_{2,51}=0.19$, $p=0.83$) upon food intake by the tadpoles (Fig. 5).

In metamorphosis experiment 1, 9 normal and 16 anomalous tadpoles completed metamorphosis. Most of the toadlets ($n=29$) were normal (78.9% from the normal group and 87.5% from the anomalous group), however,

anomalous toadlets ($n=6$) were observed from both groups (21.1% from the normal group and 12.5% from the anomalous group). All anomalies were found in the legs and feet. One individual did not develop the right leg (ectromelia), and the remaining five toadlets presented brachydactyly (reduced number of phalanges) in the left foot ($n=3$) and in both feet ($n=2$).

DISCUSSION

We showed that the presence of oral anomalies affects tadpole foraging behaviour and efficiency, since these individuals spend less time foraging and acquire less food when compared to normal tadpoles. This result is similar to that observed by Rowe et al. (1996), who found that tadpoles with oral anomalies ate less periphyton than normal ones. Tooth rows are used to both anchor the mouth and rasp surfaces during feeding (Wassersug & Yamashita, 2001; Venesky et al., 2010, 2013). We

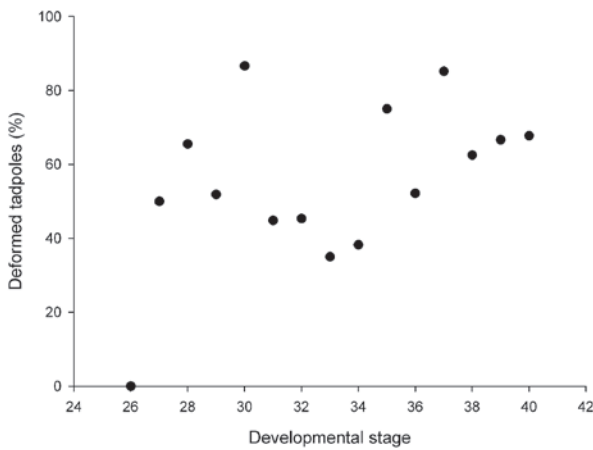


Fig. 3. Percentage of anomalous tadpoles of each stage of Gosner (1960).

measured foraging behaviour by the time spent foraging, or the time that each tadpole spends adhered to the substrate. Tadpoles with missing teeth seem to be less effective at anchoring to the substrate (Venesky et al., 2010), which could explain the pattern we found. The result of the foraging efficiency experiment is probably related to that foraging behaviour, since tadpoles that spend less time foraging would obtain less food.

In this *Rhinella jimi* population we observed 19 different types of tadpole oral anomalies, with nine of them undescribed (Drake et al., 2007; Medina et al., 2013). Unlike Drake et al. (2007) and Medina et al. (2013) who found more anomalies in A1, A2, and P2 tooth rows, we found that A1 and P3 had the higher numbers of anomalies, and in concurrence, P1 was the tooth row least affected by anomalies. Differences in the abnormal region may reflect differences of abnormality causes, but that suggestion remains understudied.

Our study, as far as we are aware, is the first to report tadpole anomalies in an introduced anuran population. These tadpoles presented an unusually high rate of anomalies (Lannoo, 2008; Medina et al., 2013), and the proportion of anomalies in tooth rows and jaw sheaths (96.7% and 3.3%, respectively) was similar to that observed by Drake et al. (2007) in closely related species, *Anaxyrus fowleri*, *A. woodhousii* and *Incilius nebulifer*. Therefore, we would summarise that the difference from natural populations is the actual rate of anomalies, not where they occur.

The proportion of anomalous tadpoles was not related to its developmental stage, unlike observed by Medina et al. (2013). In that study anomaly prevalence was lower in the later stages. Our result suggests no differential mortality caused by anomalies between stages. The negative effect caused by anomalies in feeding presented here does not necessarily result in higher mortality, but can affect the time of development and size at metamorphosis (Travis, 1984). A smaller body mass could expose individuals 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).

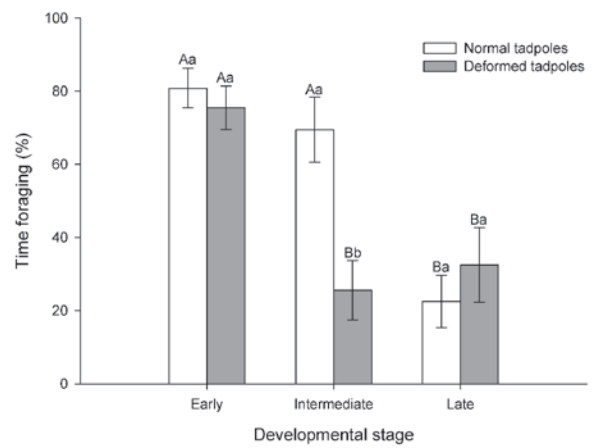


Fig. 4. Percentage of time spent foraging by normal and anomalous *Rhinella jimi* tadpoles of Fernando de Noronha archipelago, Brazil, in three different stages of development. Means and standard error bars. Early stages (26–29 of Gosner, 1960); intermediate stages (30–34 of Gosner 1960); and late stages (35–40 of Gosner 1960). Different uppercase letters represent differences between developmental stages and different lowercase letters represent differences between normal and anomalous tadpoles as indicated by a Tukey test at 5%.

Although tadpole anomalies are different from those observed in post-metamorphic individuals, the prevalence of anomalies in tadpoles (52.5%; present study) and adults (44.6%; Toledo & Ribeiro, 2009) are not statistically different. Such similarity could strengthen the hypothesis that abnormal tadpoles metamorphose into abnormal toadlets. However this was not the case, since some of the abnormal tadpoles turned into normal toadlets and also some of the normal tadpoles turned into abnormal toadlets. Therefore, there is no direct relationship between tadpole and post-metamorphic individual's anomalies. In this context we suggest that tadpole and adult anomalies are independent and there must be another trigger causing anomalies, such as individual genetic load.

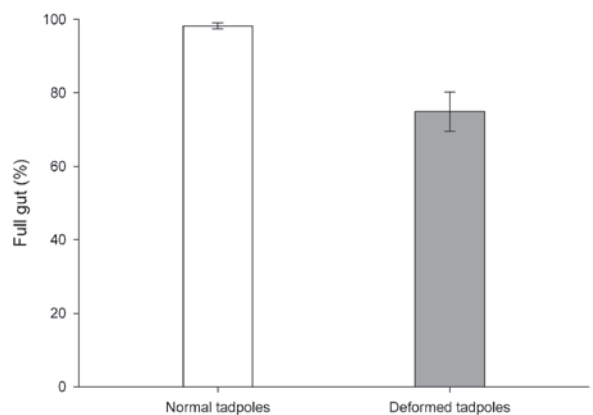


Fig. 5. Percentage of gut with food content after a three hour trial of feeding in normal and anomalous tadpoles of *Rhinella jimi* from Fernando de Noronha. Means and standard error bars shown.

We suggest some possible explanations for the maintenance of anomalous individuals in the population: i) the predator assemblage on the island is deficient (see Toledo & Ribeiro, 2009) as there are no snakes, large mammals, nocturnal birds or other large amphibians present that could prey upon and select for the handicapped tadpoles; ii) algae and other food items are so abundant in the water bodies that it is unlikely that resource competition is high; iii) there are no negative fitness effects of the smaller size at metamorphosis or later metamorphosis (not tested); and iv) as anomalous toadlets also derive from normal tadpoles, even the apparently healthier, faster-growing and larger (which would be less susceptible to predation and competition (Formanowicz, 1986; Jara & Perotti, 2010), contributes to the exceptionally high rates of adult (post-metamorphic) anomalous individuals in the population.

The presence of anomalies is common amongst natural populations, but the expected rate of anomalies in amphibians, due to mutation, errors in development and injury, is about 5% or less (Blaustein & Johnson, 2003; Lunde & Johnson, 2012). The rate of anomalies in *Rhinella jimi* tadpoles was higher than 50%, suggesting there are factors other than natural ones causing anomalies in this population. Several factors are pointed out as possible causes of amphibian anomalies, such as pesticide exposition (Bridges, 2000; Egea-Serrano et al., 2012; Agostini et al., 2013; Bacon et al., 2013), trematode infection (Johnson et al., 1999; Roberts & Dickinson, 2012), differential predation (Ballengée & Sessions, 2009; Bowerman et al., 2010), temperature during development (Bresler, 1954), UVB radiation (Blaustein et al., 1997; Ankley et al., 1998; Bacon et al., 2013), extreme tadpole density (Lannoo, 2008; Brett Sutherland et al., 2009) and salinity (Karraker, 2007). Besides these, inbreeding depression could also be a cause as it is associated with morphological anomalies in other vertebrates (e.g., lizards - Olsson et al., 1996; fish - Afonso et al., 2000; and panthers - Mansfield & Land, 2002). However, this last cause has never been reported in amphibians (Williams et al., 2008). The salinity in 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 where the rivulets meet the sea. Adults were also observed sheltering and moving in the sand on the beach, an undescribed micro-habitat for this species. Low concentrated saline water could be a favourable environment for the development of tadpoles (e.g., *Rhinella marina*, Ely, 1944), but amphibian larvae exposed to high salt concentrations can show higher rates of anomalies (e.g., Karraker, 2007). Toledo & Ribeiro (2009) suggested that the high rate of anomalies among *Rhinella jimi* populations from Fernando de Noronha could be natural. However, anomalous toads are not removed by predators, as occurs in the mainland population, because of the lack of predators on the island.

In the present study we described the abnormalities of *Rhinella jimi* tadpoles from Fernando de Noronha, tested them for possible associated handicaps and

provided some hypotheses about their causes. Our data provide valuable information about the anatomy of the morphological abnormalities and may be helpful in identifying recurring patterns. Moreover, the foraging experiments corroborate previous studies on the performance of abnormal individuals. This study complements the knowledge about the life history from an exotic anuran population, but the anomaly causes remain to be tested and warrant further study.

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