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Digestive morphology and enzyme activity in the Andean toad *Bufo spinulosus*: hard-wired or flexible physiology?

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Abstract

Gut plasticity is a trait with implications on animal performance. However, and despite their importance as study models in physiology, research on gut flexibility in amphibians is scarce. In the present work, we analyse digestive adjustments of *Bufo spinulosus* adult individuals to cope with changes in diet quality and quantity at two organizational levels (i.e., digestive morphology and enzymes). We found that changes in gut size are related to the amount of food ingested, but not to diet composition. This is in agreement with "the gut seasonal change" hypothesis and offers a proximal explanation for this change. Digestive enzymatic activity (maltase and aminopeptidase-N) did not change with diet quality or quantity, which agrees with the hypothesis of "hard-wired physiology in adult amphibians". Both hypotheses are in agreement with the general theoretical framework of gut phenotypic flexibility when interpreted in light of amphibian natural history. In addition, our results indicate that the correlation between feeding frequency and the level of gut up-regulation proposed for interspecific comparisons may also be found at the intraspecific level.

Keywords: Amphibians; *Bufo spinulosus*; Digestive theory; Digestive enzymes; Food intake; Food quality; Gastrointestinal tract; Gut size; Phenotypic flexibility

1. Introduction

A capacity for phenotypic change within genetically uniform organisms, in response to different environmental conditions, is known as phenotypic plasticity (Bradshaw, 1965; Pigliucci, 2001). A particular case of phenotypic plasticity is phenotypic flexibility, which refers to an organism's ability to modulate traits in a reversible, but not cyclic, way (Bozinovic et al., 2003; Piersma and Drent, 2003). These responses to changing conditions may include morphological, physiological and/or behavioural traits, and it is often conjectured that this plasticity increases an organism's biological performance, i.e. the adaptive plasticity hypothesis (Schmitt et al., 1999, 2003).

The digestive tract represents a functional link between foraging (energy intake) and energy management and allocation. Consequently, gut plasticity is a trait with important implications on animal performance (Hammond et al., 2001; Secor, 2001). Over the last decades, field observations and experimental laboratory studies have shown that digestive tract anatomy and function of many species are flexible, and can change in response to variation in environmental conditions (for reviews, see Piersma and Lindstrom, 1997; Starck, 1999; McWilliams and Karasov, 2001). However, most of these studies have been conducted in birds, mammals and reptiles, while research on gut flexibility in amphibians is scarce.

Evidence of gut flexibility in amphibians can be summarized into three major groups (Naya and Bozinovic,

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2004). First, field studies document that amphibians living in temperate regions show seasonal variations in gut morphology related to their feeding activity cycles (i.e. the gut seasonal change hypothesis; Larsen, 1992, p. 390). Second, laboratory studies report that, when adult individuals are exposed to different quality diets, they do not show flexibility in their enzymatic digestive activity (i.e. the hard-wired digestive physiology hypothesis; Toloza and Diamond, 1990a,b). Third, studies have demonstrated a correlation between feeding frequency of different species in the field and the magnitude by which these species up-regulate their digestive performance (Secor and Diamond, 1996; Secor, 2001).

In the present work, we studied digestive adjustments in adult individuals of *Bufo spinulosus* (Bufonidae) when faced with changes in diet quality and quantity. We analyzed responses at two organizational levels, the gross morphology of the digestive tract and associated organs (length and mass), and the activities of two digestive enzymes (maltase and oligopeptidase aminopeptidase-N). We evaluated two hypotheses: (1) Seasonal changes in gut size are due to changes in the amount of food consumption, leading us to predict an increase in gut mass and length in toads at higher rates of food intake. (2) Toads are unable to regulate their enzymatic activity in response to dietary chemical substrates and, consequently, we predict absence of modulation in their digestive enzymes when exposed to different diet qualities.

2. Materials and methods

2.1. Study organism and collection locality

We used the Andean toad, *B. spinulosus*, a species distributed between 18°S and 33°S latitude in Chile and from sea level to 4600 m.a.s.l. (Veloso and Navarro, 1988). All specimens were collected at the locality of El Tatio (22°20′S, 68°01′W, at 4300 m.a.s.l.) in the Andes range of northern Chile. El Tatio toads are more omnivorous than toads of nearby localities, feeding on small arthropods and cyanophyte algae (Nuñez et al., 1982).

2.2. Experimental design

We conducted two laboratory experiments. In the first experiment, 15 individuals were randomly assigned to one of two experimental treatments: a protein-rich diet (n=8) or a carbohydrate-rich diet (n=7) (Table 1). Animals were maintained in plastic containers of 35×25×15 cm with fresh water and stones inside. We conducted force feeding treatments by forcing animals to consume between 0.5% and 1.0% of their body mass per day. Experimental diets were prepared using casein and cornstarch as the protein and carbohydrate sources, respectively. The protein-rich diet was almost 100% carbohydrate-free, with the cornstarch being replaced by casein. The fibre content was similar in both experimental diets, being supplied by adding cellulose (Sigma-Aldrich, St. Louis, MO, USA). During the experiment, the environmental temperature was kept at 25±4 °C and the photoperiod at L/D=12:12. After 3 weeks of acclimation to diet, all animals were sacrificed and measured (body mass and snout-vent length).

Table 1
Composition of experimental diets expressed as percentage of total dry mass

	Carbohydrate-rich diet (%)	Protein-rich diet (%)
Casein	26.0	77.0
Cornstarch	52.5	1.5
Cellulose	15.0	15.0
Corn oil	6.5	6.5
Energy (kJ/g)	17.9	17.8

The results from Experiment 1 allowed us to reject the hypothesis that variations in diet quality determine changes in gut traits (see Section 3). Therefore, we decided to conduct a second experiment to evaluate the effect of the amount of ingested food on digestive performance. For this experiment, we pooled all of the data from our first experiment (force fed treatment, n=15) and compared this group with animals collected in the field and fasted for five days (starved treatment, n=16). We were unable to measure food consumption in the field, but after comparing the amount of energy ingested by force fed animals during the acclimation period (approximately 2.80 kJ/g) with published information of energy intake by species of the genus *Bufo* in the field (see Larsen, 1992, p. 382), we were confident that force fed animals constituted a high food consumption treatment.

2.3. Gut morphology and enzyme activity data

Animals were abdominal dissected and digestive organs were removed. Once supporting mesenteries had been cut, stomach and intestines were aligned along a ruler and length was measured to the nearest 0.1 cm. Liver and kidneys were also dissected, washed with Ringer's solution, dried to constant mass in an oven at 80 °C for 7 days and then weighed. After morphological determinations, the small intestine was washed with a 0.9% NaCl solution, weighed and immediately frozen in liquid nitrogen for enzyme determination. For enzyme analysis, the tissues were thawed and homogenized (30 s in an ULTRA TURRAX T25 homogenizer at maximum setting) in 20 volumes of 0.9% NaCl solution. Maltase activity (EC 3.2.1.20) was determined according to the method of Dahlqvist (1964), as modified by Martínez del Río (1990). Briefly, tissue homogenates (100 µl) were incubated at 25 °C with 100 µl of 56 mmol l^{-1} maltose solution in 0.1 M maleate/NaOH buffer, pH 6.5. After a 10-min incubation, reactions were stopped by adding 3 ml of a Glucose-Trinder stop-develop solution. Absorbance was measured at 505 nm with a spectrophotometer after 18 min at 20 °C.