

Gut size variation among *Bufo spinulosus* populations along an altitudinal (and dietary) gradient

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To date, digestive flexibility has been studied in dozens of vertebrate species. However, practically all of these works has ignored the importance of intraspecific physiological variability across populations inhabiting different habitats. Here, we compare the digestive tract gross morphology of three populations of the Andean toad (*Bufo spinulosus*), inhabiting along an altitudinal gradient and feeding on different food items. Results support a core prediction of digestive theory, i.e., intestinal length increases in parallel with the content of indigestible material in the natural diet. The present study suggest how variation in the abiotic environment associated with altitude (e.g., temperature, water availability, soil quality) can change biotic conditions (e.g., vegetation cover, prey availability), affect feeding behavior of individuals (e.g., width and composition of trophic niche), and, ultimately, individuals' digestive features (e.g., gut morphology).

Introduction

Animals continuously cope with environmental fluctuation through behavioral, physiological and structural adjustments that ensure an appropriate function (Weiner 1992). In this context, the digestive tract represents a functional link between energy intake and energy allocation, and thus, gut flexibility is considered a trait with important implications on animal performance (Secor 2001). This concept has motivated the analysis of digestive flexibility at biochemical, physiological and morphological levels in dozens of vertebrate species (for reviews see Piersma & Lindstrom 1997, Starck 1999, McWilliams &

Karasov 2001, Naya & Bozinovic 2004, Naya *et al.* 2007). Regarding amphibians, empirical evidence on digestive flexibility may be clustered in three major groups, namely the evaluation of seasonal changes in gut development, the analysis of dietary modulation of enzymes activities, and the study of the relationship between feeding habits and the magnitude of digestive performance regulation (see Naya & Bozinovic 2004, Crump & Franklin 2005, Sabat *et al.* 2005, Secor 2005).

Studies that evaluated the effect of diet changes on gut morphology have supported a core prediction of digestion theory (*sensu* Sibly 1981, Penry & Jumars 1987): the consumption

of food with high content of indigestible material results in an increase in gut dimensions. However, the validity of this assertion has been poorly analyzed at the populational level, and to date, these kind of evaluations are restricted to few rodent species. For example, it is known that individuals from populations of *Clethrionomys glareolus* and *Microtus agrestis* that consume greater amounts of plant material have a larger gut than animals from populations that mainly predate on seeds (Hansson 1985, Hansson and Jaarola 1989). Indeed, specimens of *Apodemus sylvaticus* from a seed eating population have larger small intestines than specimens from a population that largely predate on invertebrates (Corp *et al.* 1997).

Here, we compared the gross morphology of the digestive tracts of three populations of the Andean toad (*Bufo spinulosus*) in northern Chile (Fig. 1), along a gradient of altitude with a concomitant change in diet. According to Valencia *et al.* (1982) toads from low altitudes on the Chilean coast (Azapa), consume much more plant material than toads from middle (Putre) and high (Parinacota) altitudes (Table 1). In addition, these authors stated that Azapa toads exhibit a generalist diet, foraging on terrestrial prey items, whereas Putre and Parinacota toads have a more specialized diet, and tend to consume aquatic prey (Table 2). Thus, based on digestion theory, we predict greater intestinal length in the specimens consuming more plant food items (i.e., belonging to the Azapa population) than in those consuming more animal preys (i.e., belonging to the Putre and Parinacota populations).

Methods

To test our prediction we analyzed a total of 23 adult specimens: 9 toads from Azapa (4 males



Fig. 1. Map of the study area, showing the locality of each population analyzed.

and 5 females), 7 from Putre (4 males and 3 females), and 7 from Parinacota (3 males and 4 females). We used the same group of individuals studied by Valencia *et al.* (1982). The time elapsed between fixation, in 10% formalin, and dissection was the same for all three populations (12 years). Although the long-term effect of fixation on gut morphometry has not been previously investigated (Simons 2002), there is no reason to expect that a differential effect of fixation process in different populations. For each specimen snout to vent length was measured with a digital caliper (Mitutoyo; to the nearest 0.01 mm), and then animals were dissected and their intestines removed. Once supporting mesenteries had been cut, the small and large intestines were aligned

Table 1. Geographic data and diet composition for each locality (data from Valencia *et al.* 1982). %VA = volumetric percentage of arthropods, %VPM = volumetric percentage of plant material, *n* = number of stomach analyzed.

Locality	Coordinates	Altitude (m a.s.l.)	<i>n</i>	%VA	%VPM
Azapa	18°30'S, 70°13'W	164	23	61.48	36.48
Putre	18°11'S, 69°33'W	3507	18	88.14	9.86
Parinacota	18°12'S, 69°16'W	4445	24	96.56	2.91

Table 2. Diet composition, expressed as relative frequency (%) (PRF_{*i*} = [observed occurrence of item *i* / Σobserved occurrence of all items] × 100), for each population (data from Valencia *et al.* 1982). Sample sizes are given in Table 1.

	Azapa (%)	Putre (%)	Parinacota (%)
Acarina	–	–	5
Aranea	19	14	9
Coleoptera	26	19	38
Crustacea	9	–	–
Dermaptera	19	–	–
Diptera	–	19	28
Hemiptera	–	–	7
Hymenoptera	–	10	–
Lepidoptera	–	21	–
Plant material	27	17	13

along a ruler and measured to the nearest 0.1 mm.

Differences in snout to vent length, small intestine length and large intestine length among populations were evaluated separately using one-way ANOVA, with population locality as the main factor. For both small and large intestine lengths, SVL was investigated as covariate, but no significant relationships among them were found. Thus, we only report results from one-way ANOVA. We performed all analyses both with and without sex as a factor, but since sex had no effect on the measured variables, we report results without including sex. Prior to analyses, data were tested for a normal distribution and homogeneity of variances. Data on large intestine length were log-transformed to meet ANOVA assumptions. Pair-wise comparisons among localities were conducted using the Tukey HSD test for unequal sample sizes. Statistical significance was established at the 0.05 level, and probability values between 0.05 and 0.1 were considered as marginally significant. Statistical analyses were performed using the

statistical package, STATISTICA® version 6.0 for Windows®.

Results

Snout to vent length differed among populations (Table 3). Pair-wise comparisons revealed that toads from Parinacota were smaller than those from Azapa ($p = 0.01$) and Putre ($p = 0.03$). Differences in small intestine length among populations were also significant (Table 3), and pair-wise comparisons indicated that animals from Azapa had significantly longer intestines than animals from Parinacota ($p = 0.01$), and marginally longer intestines than animals from Putre ($p = 0.06$). Large intestine length follows the same pattern of variation than the small intestine length, but in this case only a marginal probability was reached (Table 3). *A posteriori* comparisons indicate that Azapa's toads tend to show greater large intestine length than those from Parinacota ($p = 0.09$). Note that recorded differences in gut length among populations did not follow the same pattern of variation than body size, which precludes the possibility that any differential effect of body mass on intestinal tissue fixation affected our results.

Discussion

Most of the current knowledge on digestive flexibility comes from two particular situations: (1) adjustments of small endotherms (mainly rodent and birds) to cope with changes in ambient temperature, food quality and/or reproductive status, and (2) adjustments of middle-size and large ectotherms (mainly snakes) after a meal ingestion. However, in recent years, an increasing number of studies on digestive flex-

Table 3. Snout to vent length (SVL), small intestine length (SIL) and large intestine length (LIL) for each population. Values reported are absolute means (± SE).

Trait	Azapa ($n = 9$)	Putre ($n = 7$)	Parinacota ($n = 7$)	F and p
SVL (mm)	72.9 (± 1.4)	71.1 (± 1.5)	64.9 (± 1.4)	$F_{2,20} = 8.04, p < 0.01$
SIL (mm)	85.6 (± 4.5)	68.1 (± 5.1)	61.9 (± 5.1)	$F_{2,20} = 6.68, p < 0.01$
LIL (mm)	24.1 (± 1.8)	22.2 (± 2.1)	17.6 (± 2.1)	$F_{2,20} = 2.72, p = 0.09$

ibility in small ectotherm vertebrates have been published (e.g., Secor 2001, 2005, Crump & Franklin 2003, 2005, Sabat *et al.* 2005, Naya & Bozinovic 2006). Indeed, these studies demonstrated that these organisms are able to adjust their digestive traits in response to changes in external conditions.

Results obtained here support a core prediction of the digestion theory; that is, specimens feeding on plant material (Azapa) have greater intestinal length than animals preying on animal (Putre and Parinacota). Similar results were previously obtained by Nuñez *et al.* (1982), who found that *Bufo spinulosus* individuals from a population that mainly consume Cyanophyta algae (El Tatio) had larger intestines than toads from a population that mainly consume arthropods (San Pedro de Atacama). Two lines of evidences suggest that the observed differences in gut length among populations could be due, at least in part, to phenotypic plasticity. First, studies on genetic differentiation of *B. spinulosus* in Chile demonstrate that there are low levels of genetic variation among the studied populations (Mendez *et al.* 2004). Second, a previous experimental work conducted on *B. spinulosus* demonstrated that intestinal length is sensible to changes in the amount of food ingested; specifically, it was found that feeding toads have greater gut size than fasted animals (Naya *et al.* 2005). However, to determine if the observed differences among populations are due to phenotypic flexibility, local adaptation, or both process acting together, requires further tests (e.g., common garden experiments).

Studies regarding digestive flexibility in nature have focused on seasonal changes within populations, and there are very few reports on gut variation among populations (but *see* Hansson 1985, Corp *et al.* 1997, Tracy & Diamond 2005). Nevertheless, studies that compare populations from different habitats are crucial for understanding how physiological variables are affected by environmental conditions, and consequently, how they evolve (Spicer & Gaston 1999, Chown & Nicolson 2004). In this sense, the present study suggest how variation in the abiotic environment associated with altitude (e.g., temperature, water availability, soil quality) can change biotic conditions (e.g., vegetal

cover, prey availability), affect feeding behavior of individuals (e.g., trophic niche width, consumption of terrestrial or aquatic prey), and, ultimately, individuals' digestive performance (e.g., gut morphology).

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References

- Corp, N., Gorman, M. L. & Speakman, J. R. 1997: Apparent absorption efficiency and gut morphometry of wood mice, *Apodemus sylvaticus*, from two distinct populations with different diets. — *Physiological Zoology* 70: 610–614.
- Chown, S. L. & Nicolson, S. W. 2004: *Insect physiological ecology*. — Oxford University Press, New York.
- Crump, R. L. & Franklin, C. E. 2003: Is re-feeding efficiency compromised by prolonged starvation during aestivation in the green striped burrowing frog, *Cyclorana albogutata*. — *Journal of Experimental Zoology* 300A: 126–132.
- Crump, R. L. & Franklin, C. E. 2005: Arousal and re-feeding rapidly restores digestive tract morphology following aestivation in green-striped burrowing frogs. — *Comparative Biochemistry and Physiology A* 142: 451–460.
- Hansson, L. 1985: Geographic differences in bank voles *Clethrionomys glareolus* in relation to ecogeographical rules and possible demographic and nutritive strategies. — *Annales Zoologici Fennici* 22: 319–328.
- Hansson, L. & Jaarola, M. 1989: Body size related to cyclicality in microtines: dominance behaviour or digestive efficiency? — *Oikos* 55: 396–364.
- McWilliams, S. R. & Karasov, W. H. 2001: Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. — *Comparative Biochemistry and Physiology A* 128: 579–593.
- Mendez, M. A., Soto, E. R., Correa, C., Veloso, A., Vergara, E., Sallaberry, M. & Iturra, P. 2004: Morphological and genetic differentiation among Chilean populations of *Bufo spinulosus* (Anura: Bufonidae). — *Revista Chilena de Historia Natural* 77: 559–567.
- Naya, D. E. & Bozinovic, F. 2004: Digestive phenotypic flexibility in post-metamorphic amphibians: studies of a model organism. — *Biological Research* 37: 365–370.
- Naya, D. E. & Bozinovic, F. 2006: The role of ecological interactions on the physiological flexibility of lizards. — *Functional Ecology* 20: 601–608.
- Naya, D. E., Farfán, G., Sabat, P., Mendez, M. A. & Bozinovic, F. 2005: Digestive performance in the Andean toad *Bufo spinulosus*: flexibility or hard-wired physiol-

- ogy? — *Comparative Biochemistry and Physiology A* 140: 165–170.
- Naya, D. E., Karasov, W. H. & Bozinovic, F. 2007: Phenotypic plasticity in laboratory mice and rats: a meta-analysis of current ideas on gut size flexibility. — *Evolutionary Ecology Research* 9: 1363–1374.
- Núñez, H., Labra, M. A. & Yañez, J. 1982: Hábitos alimentarios de dos poblaciones andinas de *Bufo spinulosus* Wiegmann, 1835. — *Boletín del Museo Nacional de Historia Natural de Chile* 39: 81–89.
- Penry, D. L. & Jumars, P. A. 1987: Modeling animal guts as chemical reactors. — *American Naturalist* 129: 69–96.
- Piersma, T. & Lindstrom, A. 1997: Rapid reversible changes in organ size as a component of adaptative behaviour. — *Trends in Ecology and Evolution* 12: 134–138.
- Sabat, P., Riveros, J. M. & López-Pinto, C. 2005: Phenotypic flexibility in the intestinal enzymes of the African clawed frog *Xenopus leavis*. — *Comparative Biochemistry and Physiology A* 140: 135–139.
- Secor, S. M. 2001: Regulation of digestive performance: a proposed adaptive response. — *Comparative Biochemistry and Physiology A* 128: 565–577.
- Secor, S. M. 2005: Physiological responses to feeding, fasting and estivation for anurans. — *Journal of Experimental Biology* 208: 2595–2608.
- Sibly, R. M. 1981: Strategies of digestion and defecation. — In: Townsend, C. R. & Calow, P. (eds.), *Physiological ecology: an evolutionary approach to resource use*: 109–139. Blackwell Scientific Publications, Oxford.
- Simons, J. E. 2002: *Herpetological collecting and collections management*. — Herpetological Circular No. 31 (SSAR).
- Spicer, J. I. & Gaston, K. J. 1999: *Physiological diversity and its ecological implications*. — Blackwell Science, Oxford.
- Starck J. M. 1999: Structural flexibility of the gastro-intestinal tract of vertebrates — implications for evolutionary morphology. — *Zoologischer Anzeiger* 238: 87–101.
- Tracy, C. R. & Diamond, J. 2005: Regulation of gut function varies with life-history traits in chuckwallas (*Sauromalus obesus*: Iguanidae). — *Physiological and Biochemical Zoology* 78: 469–481.
- Valencia, J., Veloso, A. & Sallaberry, M. 1982: Nicho trófico de las especies de herpetozoos del transecto Arica-Chungará. — In: Veloso, A. & Bustos-Obregón, E. (eds.), *El ambiente natural y las poblaciones humanas de los Andes del norte grande de Chile (Arica, Lat. 18°28'S)*: 269–291. Proyecto MAB-6, UNEP-UNESCO 1105-77-01, Montevideo.
- Wiener, J. 1992: Physiological limits to sustainable energy budgets in birds and mammals: ecological implications. — *Trends in Ecology and Evolution* 7: 384–388.