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

Daniel E. Naya[a], Gonzalo Farfán[b,†], Pablo Sabat[a,b], Marco A. Méndez[c], Francisco Bozinovic[a,*]

[a]*Center for Advanced Studies in Ecology and Biodiversity and Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago 6513677, Chile*
[b]*Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile*
[c]*Laboratorio de Bioinformática y Expresión Génica, INTA; Universidad de Chile, Santiago, Chile*

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,

* Corresponding author. Tel.: +562 686 2618; fax: +562 686 2621.
E-mail address: fbozinov@bio.puc.cl (F. Bozinovic).
† Deceased.

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.

Aminopeptidase-N (EC 3.4.11.2) assays were conducted with L-alanine-*p*-nitroanilide as a substrate. Briefly, 100 μl of homogenate diluted with 0.9% NaCl solution was mixed with 1 ml of assay mix (2.04 mmol l^{-1} L-alanine-*p*-nitroanilide in 0.2 mol μl^{-1} NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated at 25 °C and stopped after 10 min with 3 ml of ice-cold acetic acid (2 N) and absorbance was measured at 384 nm. The selected pH for measuring the activities were the optimum for each enzyme. The activity of enzymes is tabulated as enzyme activity rate (UI per g wet intestine, where UI=μmol substrate hydrolyzed per min) and total enzyme activity (for a detailed explanation, see Martínez del Rio et al., 1995).

2.4. Statistical analyses

Differences in body mass and snout-vent length between groups were tested separately by a one-way ANOVA. Changes in all digestive variables were evaluated separately by ANCOVA, using the snout-vent length or body mass (choosing the best predictor variable in each analysis) as the covariable. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. Interactions between covariables and factors were also tested (parallelism test). When necessary to meet assumptions, variables were transformed to the inverse or to the square root. Relationships between enzyme activities were evaluated using the Pearson product-moment correlation coefficient. Statistical analyses were performed using

Table 2
Body size, morphology and enzymatic activity data for the two diet quality treatments, expressed as least square adjusted means (±S.E.)

	Protein-rich diet (n=7)	Carbohydrate-rich diet (n=8)	F and P values
Body mass (g)	6.72 (1.37)	6.83 (1.46)	$F_{1,13}$=0.003, P=0.96
Snout-vent length (cm)	3.86 (0.30)	3.97 (0.32)	$F_{1,13}$=0.06, P=0.81
Length (cm)			
Stomach	1.48 (0.04)	1.52 (0.04)	$F_{1,12}$=0.42, P=0.53
Small intestine	7.86 (1.00)	8.58 (1.07)	$F_{1,12}$=0.24, P=0.63
Large intestine	1.31 (0.11)	1.42 (0.12)	$F_{1,12}$=0.56, P=0.47
Wet mass (g)			
Small intestine	0.18 (0.02)	0.18 (0.02)	$F_{1,12}$=0.02, P=0.89
Dry mass (g)			
Liver	0.34 (0.04)	0.24 (0.04)	$F_{1,12}$=3.29, P=0.09
Kidneys	0.05 (0.003)	0.05 (0.004)	$F_{1,12}$=0.03, P=0.85
Activity rate (UI g^{-1})			
Maltase	1000.7 (142.2)	776.1 (152.0)	$F_{1,12}$=1.27, P=0.28
Aminopeptidase-N	359.3 (58.8)	320.6 (62.9)	$F_{1,12}$=0.20, P=0.66
Total activity (UI)			
Maltase	158.3 (19.2)	142.9 (20.6)	$F_{1,12}$=0.30, P=0.59
Aminopeptidase-N	64.8 (13.8)	61.7 (14.7)	$F_{1,12}$=0.02, P=0.88

Table 3
Body size and enzymatic activity data for starved and force fed animals, expressed as least square adjusted means (±S.E.)

	Starved (n=16)	Force fed (n=15)	F and P values
Body mass (g)	4.85 (0.86)	6.77 (0.89)	$F_{1,29}$=2.32, P=0.14
Snout-vent length (cm)	3.64 (0.23)	3.91 (0.23)	$F_{1,29}$=0.69, P=0.41
Activity rate (UI g^{-1})			
Maltase	1090.1 (97.3)	884.3 (100.4)	$F_{1,28}$=2.09, P=0.16
Aminopeptidase-N	485.3 (67.3)	350 (65.0)	$F_{1,28}$=2.04, P=0.16
Total activity (UI)			
Maltase	122.1 (10.8)	131.9 (11.2)	$F_{1,28}$=0.39, P=0.54
Aminopeptidase-N	44.1 (8.3)	57.9 (8.5)	$F_{1,28}$=1.31, P=0.26

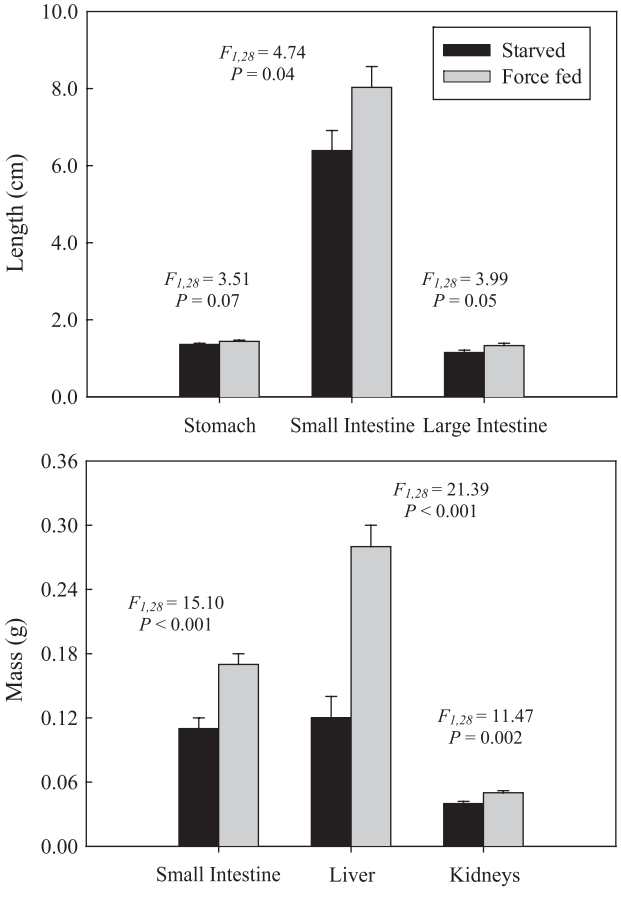

Fig. 1. Morphology data for starved and force fed groups expressed as least square adjusted means (±S.E.). Small intestine mass refers to wet mass.

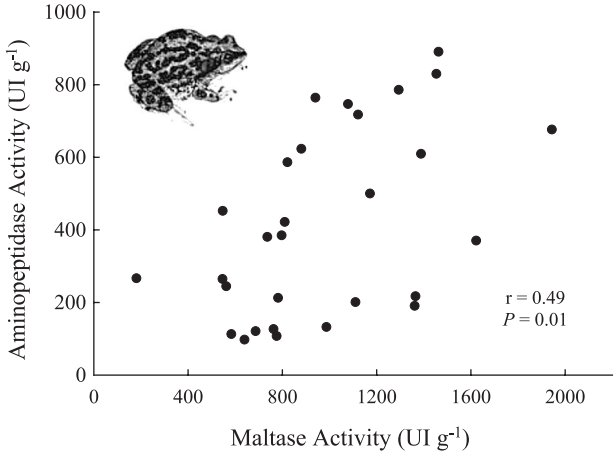

Fig. 2. Correlation between intestinal maltase and aminopeptidase-N activities.

the statistical package, STATISTICA® (2001) version 6.0 for the Windows® operating system.

3. Results

3.1. Experiment 1: effect of diet quality

No significant differences were noted in body mass or snout-vent length between protein and carbohydrate dietary treatments (Table 2). Similarly, no changes in morphology or enzymatic activity were recorded between treatments (Table 2).

3.2. Experiment 2: effect of diet quantity

There were no significant differences in body mass or snout-vent length between starved and force fed animals (Table 3). Morphology data showed a clear pattern of size increase in digestive and associated organs in force fed animals (Fig. 1). This trend was more apparent for organ mass ($P \leq 0.002$ for all comparisons) than for length. Indeed, stomach length and large intestine length only presented marginal probabilities between treatment groups ($P=0.07$ and 0.05, respectively).

Regarding enzymatic analyses, we did not observe significant variation in activity rate or total activity between groups for either of the two digestive enzymes evaluated (Table 3). We found a significant positive correlation between maltase and aminopeptidase-N activities (Fig. 2).

4. Discussion

Amphibians have played a key role as a study model in the development of modern physiology and, during the past century, this group has become a standard model for the study of several biological processes (Feder, 1992). From the perspective of digestive physiology, amphibians have several characteristics that make them a highly interesting group, such as their adaptation to low energy flow (sensu Pough, 1980; see also Secor, 2001; Naya and Bozinovic, 2004).

Nevertheless, as we pointed out earlier, studies dealing with digestive flexibility in amphibians are scarce. Based on an early study of seasonal variation in gut mass of *Rana temporaria* (Juszczyk et al., 1966) and some circumstantial evidence (e.g., Herter, 1936; Geuze, 1971a,b), Larsen (1992) proposed the gut seasonal change hypothesis for amphibians. This hypothesis states that seasonal changes in gut size occur (at least in species inhabiting temperate zones) and greater gut sizes are expected to be observed during periods of high feeding activity. Recently, an analysis of seasonal variation in the intestinal length of the South American common frog (*Leptodactylus ocellatus*) reinforced the existence of seasonal changes related to feeding events (Naya et al., 2003).

As far as we know, the present study is the first to experimentally evaluate the effect of diet quality and quantity on digestive morphology in adult amphibians. We found that changes in gut size are related to the amount of food ingested, but not to the dietary chemical composition. These results support the seasonal change hypothesis and offer a proximal explanation, namely seasonal changes in gut size are due to changes in the amount of food ingested. In addition, our results agree with the interspecific correlation between feeding frequency and the levels of gut up-regulation (Secor and Diamond, 1996; Secor, 2001), suggesting that this pattern can also be found at the intraspecific level (i.e., among populations with different feeding frequencies).

Regarding digestive enzymes, the adaptive modulation hypothesis (Diamond, 1991; Karasov, 1992) states that the expression of digestive proteins should be modulated in response to the ingestion of their respective substrates. However, dietary modulation of enzymatic activity is by no means a universal fact (Sabat et al., 1998). In this sense, current data for amphibians indicate that digestive enzymes and brush border transporters in adult individuals are not adjusted to changes in diet composition (Toloza and Diamond, 1990a,b; Sabat and Bozinovic, 1996). This hypothesis, called the hard-wired hypothesis, is also supported by our results, since we neither found changes in activity between both dietary groups nor between force fed and starved animals for the enzymes tested. Interestingly, specific enzyme activity seems not to be affected by the fasting condition, while gross morphology is affected. It is likely that the atrophy of digestive organs, particularly the small intestine, is not coupled with the disintegration of villus of the intestinal cells, as has been demonstrated in birds (Karasov et al., 2004), or with the probable decrease of biochemical capacities, as reported in reptiles (Secor and Diamond, 1995). This idea suggests that fasted toads minimize the cost of maintenance of unused tissues, but

maintain a biochemical machinery to digest and probably to efficiently absorb nutrients in unpredictable future feeding events. Another interesting result is the correlation observed between maltase and aminopeptidase-N activities. Although this has also been reported for other species (Sabat et al., 1998), to our knowledge, an explanation for this puzzling phenomenon is still lacking.

In summary, phenotypic flexibility is often hypothesized to be responsible for allowing organisms to adjust to changing biotic and abiotic environmental conditions through increases in biological performance. We found evidence supporting both the gut seasonal change hypothesis and the hard-wired physiology hypothesis. These two apparently contrasting ideas are not contradictory if one considers that: (a) species living in temperate regions likely face strong seasonal variation in abiotic and biotic factors; thus, seasonal variations in gut dimensions are likely (Larsen, 1992; Piersma and Lindstrom, 1997); and (b) food composition in adult anurans varies slightly (Toloza and Diamond, 1990b), hence, a lack of dietary modulation of digestive enzymes in adults is also expected (Buddington et al., 1991; Toloza and Diamond, 1990b). In other words, both hypotheses are in agreement with the general theoretical framework of digestive phenotypic flexibility (e.g. Sibly, 1981; Penry and Jumars, 1987; Martínez del Río et al., 1994) when interpreted in light of amphibian natural history.

Acknowledgments

DEN acknowledge a fellowship from the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT, Chile). This work was funded by Fondo Nacional de Ciencia y Tecnología FONDAP 1501-0001 (Program 1) to FB, FONDECYT 3000048 to MAM and FONDECYT 1010647 to PS. All experiments were conducted according to current Chilean law and under permit 698 from SAG. This paper is dedicated to the memory of our dear student and friend, Gonzalo "Conejo" Farfán.

References

Bozinovic, F., Gallardo, P.A., Visser, G.H., Cortés, A., 2003. Seasonal acclimatization in water flux rate, urine osmolality and kidney water channels in free-living degus: molecular mechanisms, physiological processes and ecological implications. J. Exp. Biol. 206, 2959–2966.

Bradshaw, A.D., 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13, 115–155.

Buddington, R.K., Chen, J.W., Diamond, J.M., 1991. Dietary regulation of intestinal brush-border sugar and amino acid transport in carnivores. Am. J. Physiol. 261, R793–R801.

Dahlqvist, A., 1964. Method for assay of intestinal disaccharidases. Ann. Biochem. 7, 18–25.

Diamond, J.M., 1991. Evolutionary design of intestinal nutrient absorption: enough but not too much. News Physiol. Sci. 6, 92–96.

Feder, M.E., 1992. A perspective on environmental physiology of the amphibians. In: Feder, M.E., Burggren, W.W. (Eds.), Environmental Physiology of the Amphibians. The University of Chicago Press, Chicago, pp. 1–6.

Geuze, J.J., 1971a. Light and electron microscope observations on the gastric mucosa of the frog *Rana esculenta*. I. Normal structure. Z. Zellforsch. 117, 87–102.

Geuze, J.J., 1971b. Light and electron microscope observations on the gastric mucosa of the frog (*Rana esculenta*): II. Structural alteration during hibernation. Z. Zellforsch. 117, 103–117.

Hammond, K.A., Szewczak, J., Król, E., 2001. Effects of altitude and temperature on organ phenotypic plasticity along an altitudinal gradient. J. Exp. Biol. 204, 1991–2000.

Herter, K., 1936. Die Physiologie der Amphibien. In: Kükenthal, W. (Ed.), Handbuch der Zoologie. de Gruyter, Berlin. 252 pp.

Juszczyk, W., Obrzut, K., Zamachowski, W., 1966. Morphological changes in the alimentary canal of the common frog (*Rana temporaria* L.) in the annual cycle. Acta Biol. Crac., Ser. Zool. IX, 239–246.

Karasov, W.H., 1992. Test of the adaptive modulation hypothesis for dietary control of intestinal transport. Am. J. Physiol. 263, R496–R502.

Karasov, W.H., Pinshow, B., Starck, J.M., Afik, D., 2004. Anatomical and histological changes in the alimentary tract of migrating blackcaps (*Sylvia atricapilla*): a comparison among fed, fasted, food-restricted, and refed birds. Physiol. Biochem. Zool. 77, 149–160.

Larsen, L.O., 1992. Feeding and Digestion. In: Feder, M.E., Burggren, W.W. (Eds.), Environmental Physiology of the Amphibians. The University of Chicago Press, Chicago, pp. 378–394.

Martínez del Río, C., 1990. Dietary and phylogenetic correlates of intestinal sucrose and maltase activity in birds. Physiol. Zool. 63, 987–1011.

Martínez del Río, C., Cork, S.J., Karasov, W.H., 1994. Modelling gut function: an introduction. In: Chiver, J., Langer, P. (Eds.), The Digestive System in Mammals. Cambridge University Press, Cambridge, pp. 25–53.

Martínez del Rio, C., Brugger, K., Witmer, M., Rios, J., Vergara, E., 1995. An experimental and comparative study of dietary modulation of intestinal encimes in European starling (*Sturnus vulgaris*). Physiol. Zool. 68, 490–511.

McWilliams, S.R., Karasov, W.H., 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comp. Biochem. Physiol., A 128, 579–593.

Naya, D.E., Bozinovic, F., 2004. Digestive phenotypic flexibility in post-metamorphic amphibians: studies on a model organism. Biol. Res. 37, 365–370.

Naya, D.E., Maneyro, R., Camargo, A., Da Rosa, I., Canavero, A., 2003. Annual changes in gut length of the South American common frog (*Leptodactylus ocellatus*). Biociencias 11, 47–52.

Nuñez, H., Labra, M.A., Yáñez, J., 1982. Hábitos alimentarios de dos poblaciones andinas de *Bufo spinulosus* Wiegmann, 1835 (Anura: Bufonidae). Bol. Mus. Nac. Hist. Nat. Chile 39, 81–91.

Penry, D.L., Jumars, P.A., 1987. Modeling animal guts as chemical reactors. Am. Nat. 129, 69–96.

Piersma, T., Drent, J., 2003. Phenotypic flexibility and the evolution of organismal design. Trends Ecol. Evol. 18, 228–233.

Piersma, T., Lindstrom, A., 1997. Rapid reversible changes in organ size as a component of adaptative behaviour. Trends Ecol. Evol. 12, 134–138.

Pigliucci, M., 2001. Phenotypic Plasticity: Beyond Nature and Nurture. Johns Hopkins University Press. 384 pp.

Pough, F.H., 1980. Amphibians and reptiles as low-energy systems. In: Wayne, P.A., Lustick, S.I. (Eds.), Behavioral Energetics: the Cost of Survival in Vertebrates. Ohio State University Press, Columbus, pp. 141–188.

Sabat, P., Bozinovic, F., 1996. Dietary chemistry and allometry of intestinal disaccharidases in the toad *Bufo spinulosus*. Rev. Chil. Hist. Nat. 69, 387–391.

Sabat, P., Novoa, F., Bozinovic, F., Martínez del Rio, C., 1998. Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. Physiol. Zool. 71, 226–236.

Schmitt, J., Dudley, S.A., Pigliucci, M., 1999. Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. Am. Nat. 154, S43–S54.

Schmitt, J., Stinchcombe, J.R., Heschel, M.S., Huber, H., 2003. The adaptive evolution of plasticity: phytochrome-mediated shade avoidance responses. Integr. Comp. Biol. 43, 459–469.

Secor, S.M., 2001. Regulation of digestive performance: a proposed adaptive response. Comp. Biochem. Physiol., A 128, 565–577.

Secor, S.M., Diamond, J.M., 1995. Adaptive responses to feeding in Burmese pythons: pay before pumping. J. Exp. Biol. 198, 1313–1325.

Secor, S.M., Diamond, J.M., 1996. Adaptive responses of digestive physiology in frogs. Am. Zool. 36, 17A.

Sibly, R.M., 1981. Strategies of digestion and defecation. In: Towsend, C.R., Calow, P. (Eds.), Physiological Ecology: An Evolutionary Approach to Resource Use. Blackwell Scientific Publications, Oxford, pp. 109–139.

Starck, M., 1999. Structural flexibility of the gastro-intestinal tract of vertebrates—implications for evolutionary morphology. Zool. Anz. 238, 87–101.

Toloza, E.M., Diamond, J.M., 1990a. Ontogenetic development of nutrient transporters in bullfrog intestine. Am. J. Physiol. 258, G760–G769.

Toloza, E.M., Diamond, J.M., 1990b. Ontogenetic development of transporters regulation in bullfrog intestine. Am. J. Physiol. 258, G770–G773.

Veloso, A., Navarro, J., 1988. Lista sistemática y distribución geográfica de anfibios y reptiles de Chile. Boll.-Mus. Reg. Sci. Nat. 6, 481–539.