



Physiological flexibility and climate change: The case of digestive function regulation in lizards

Daniel E. Naya^{a,b,*}, Claudio Veloso^c, Pablo Sabat^{b,c}, Francisco Bozinovic^b

^a Sección Evolución - Facultad de Ciencias and Centro Universitario de la Regional Este, Universidad de la República, Montevideo, Uruguay

^b Center for Advanced Studies in Ecology & Biodiversity and Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

^c Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

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ABSTRACT

Our planet is undergoing fast environmental changes, which are referred as global change. In this new scenario, it is of paramount relevance to understand the mechanistic basis of animal responses to environmental change. Here we analyze to what extent seasonal changes in the digestive function of the lizard *Liolaemus moradoensis* is under endogenous (i.e., hard wired) or exogenous (i.e., environmentally determined) control. For this purpose we compared animals collected in the field during autumn, winter and summer, against (experimental) specimens collected in the field at the beginning of autumn and reared in the laboratory under simulated summer conditions until winter. We found that different aspects of the digestive function are under different types of control: small intestine length appears to be under endogenous control (i.e., experimental animals were similar to winter animals), small intestine mass appears to be under exogenous control (i.e., experimental animals were similar to summer animals), and specific enzyme activities did not change throughout the year. Thus, we suspect that processes related with gut length, such as cell division, may be under endogenous control, while others related with gut mass, such as enterocyte size and content, may be determined by exogenous factors, such as the presence of food in the intestinal lumen. Faced with accelerated changing conditions, the ability of vertebrates to cope will be closely related with their plasticity in fitness-associated traits. More studies aimed at determining the levels and limits of physiological flexibility will be necessary to understand this phenomenon.

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1. Introduction

Our planet is undergoing dramatic environmental changes, which are referred as global change (Gaston and Spicer, 2004). It is defined as alterations in the global environment, including modifications in climate, land productivity, oceans or other water resources, atmospheric chemistry, and ecological systems, which may affect the capacity of Earth to sustain life (U.S. Global Change Research Act, 1990). In this scenario of rapid environmental alterations, it is of paramount relevance to understand different aspects of the mechanistic basis of animal responses to changing environmental conditions (Helmuth et al., 2005). One of these fundamental aspects is to determine to what extent seasonal phenotypic adjustments are under endogenous (i.e., hard wired) or exogenous (i.e., environmentally determined) control. This is because if phenotypic changes are endogenously controlled (i.e., uncoupled to an external cue) or coupled to a cue that is not affected by current environmental changes (e.g., photoperiod), a progressive

mismatch between them and their environment could be expected (Stenseth and Mysterud, 2002; Booth et al., 2006).

In animals, the digestive tract represents the functional link between energy intake and energy available for survival, growth, and reproduction (Secor, 2001). Consequently, gut flexibility could be considered a trait with important implications on overall animal performance (Naya et al., 2008). In agreement with this theoretical expectation, the digestive system has been demonstrated to be one of the most responsive to environmental conditions (Pennisi, 2005). Further, seasonal changes in digestive attributes—at several organizational levels—have been repeatedly reported during the last five decades for several vertebrates groups, including amphibians (e.g. Juszczuk et al., 1966), reptiles (e.g. Latif et al., 1967), mammals (e.g. Myrcha, 1964) and birds (e.g. Davis, 1961). But, beyond this evidence, a central question that remains is whether seasonal adjustments in digestive function are endogenously- or exogenously-controlled.

In an early paper, Juszczuk et al. (1966) suggested—based on the fact that gut size down-regulation in *Rana temporaria* starts before the beginning of the inactivity period—that there is a “physiological clock” that regulates the function of the alimentary system (i.e., an endogenous control). More recently, Larsen (1992, p. 390) used the same study together with other circumstantial evidence, to state that seasonal changes in gut function of species inhabiting temperate areas are

* Corresponding author at: Sección Evolución, Facultad de Ciencias, Universidad de la República, Iguá 4225, Montevideo 11400, Uruguay. Tel.: +598 2 5258618; fax: +598 2 5258617.

E-mail address: dnaya@fcien.edu.uy (D.E. Naya).

“basically endogenous and set by long-term adaptation to climate, and now only modified by unpredictable variations in temperature, amount of prey, and so forth.” Nevertheless, practically half a century after the proposal of seasonal changes in digestive traits, no experimental studies aimed at testing the predictions derived from this hypothesis exist.

In the present work we analyze whether seasonal changes in small intestine size and digestive enzyme activities are subject to endogenous or exogenous regulation in the Andean lizard *Liolaemus moradoensis*. For this purpose we compared animals collected in the field during autumn, winter and summer against specimens collected in the field at the beginning of autumn and reared in the laboratory under simulated summer conditions until winter. We predicted that if digestive function is under endogenous control, animals in the experimental group should have similar values to winter animals, that is, to animals analyzed at the same time of the year (Fig. 1A). By contrast, if digestive function control is exogenous, animals in the experimental group should be similar to summer specimens, that is, to animals experiencing similar environmental conditions (Fig. 1B).

2. Material and methods

2.1. Animal collection and experimental design

Adult males of the lizard *L. moradoensis* were collected by hand in the locality of Lagunillas (33° 36' S, 70° 17' W; 2370 m above sea level), in the Andean Mountains of central Chile. In this study area, lizards are highly active during spring and summer (i.e., October–November to March), with activity decreasing by mid autumn (i.e. April and May). During winter months (i.e., June to September–October) the site is covered by snow and lizards hibernate in

subnivean habitats. After collection, specimens were transferred to the laboratory and housed in individual cages (15 × 30 × 20 cm). Body mass (m_b) of each individual was measured using an electronic balance (Sartorius GmbH, Göttingen, Germany; ±0.1 g), and snout-to-vent length (SVL) was measured using a plastic ruler (±1 mm).

Our experimental design contemplates four groups (Table 1): two field-caught groups (Autumn, $n=10$; Summer, $n=10$), one group that simulates winter conditions (Winter, $n=10$), and one experimental group in which laboratory conditions were uncoupled from field conditions (Exp, $n=11$). In the two field groups animals were maintained for one day in the laboratory (without access to food) and then sacrificed. The Winter group was formed with specimens collected in April 2009 and maintained (without access to food) in climatic chambers until July 2009, under conditions that simulate progressive transition toward winter (see Appendix A). We did this because during winter months lizards are in subnivean habitats and thus cannot be collected directly from the field. However, data on small intestine length and dry mass for *Liolaemus bellii*, a species closely related to *L. moradoensis*, indicate that lizards subjected to the same winter protocol used here did not differ from animals collected in the field at the end of winter (D.E. Naya, unpublished data). Finally, the experimental group was formed with animals also collected in April 2009, but maintained in the laboratory (with access to food) under simulated summer conditions until July 2009 (Table 1). Lizards in this last group were also fasted for one day before their sacrifice.

2.2. Morphological determinations

Lizards were cooled by decreasing ambient temperature (to 0 °C), and then sacrificed through decapitation. After that, specimens were dissected and the small intestine was removed and washed with 0.9% NaCl solution, lightly dried with paper towel and weighed (Chyo JK180, Kyoto, Japan; ±0.0001 g). Then, a 2 cm section from the anterior portion of the small intestine was cut, weighed, and immediately frozen at –80 °C for subsequent measurement of digestive enzyme activity. Finally, the remaining small intestine was dried together with the animal's carcass at 60 °C for one week. We calculated small intestine dry mass as the wet mass multiplied by the dry-mass/wet-mass ratio, estimated from the small intestine section that was not used for enzymatic determination.

2.3. Enzyme activity determinations

We analyzed one disaccharidase (maltase; EC 3.2.1.20) and one oligopeptidase (aminopeptidase-N; EC 3.4.11.2) as indicators of digestive capacity of carbohydrates and proteins, respectively (Vonk

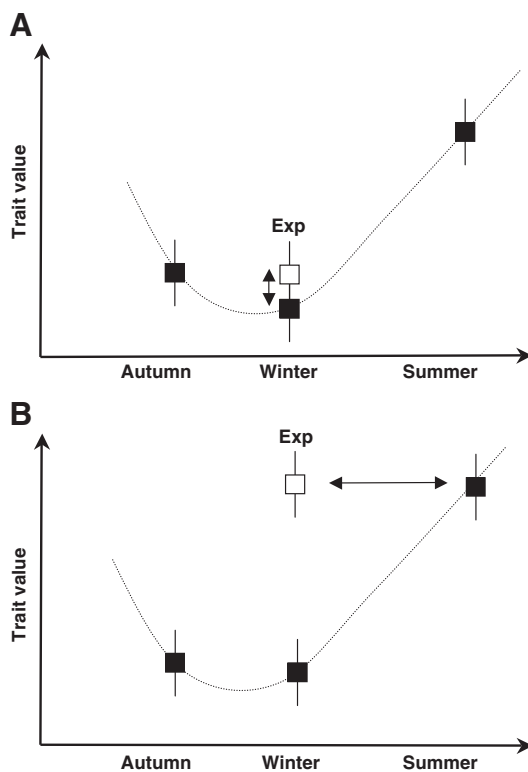


Fig. 1. Graphical representation of the hypotheses being tested. Under the endogenous control hypothesis we predict that animals in the experimental group will be similar to winter animals (A), while under the exogenous control hypothesis we predicted that the experimental group will be similar to the Summer group (B).

Table 1

Specimen collection dates (CD) and analysis (AD), sample size (N), and environmental conditions for each group used in the experiments.

	CD	AD	N	Environmental conditions
Autumn	April '09	April '09	10	Natural (daily cycles from 3.8 ± 1.9 °C to 15.2 ± 4.8 °C, photoperiod 11 L:13D)*
Winter	April '09	July '09	10	Progressive reduction in temperature and photoperiod from autumn natural conditions to 2 °C and 0L:24D (see Appendix A); lizards did not have access to food during this period
Exp	April '09	July '09	11	Temperature daily cycles from 18 to 30 °C, photoperiod 14L:10D; water and food (mealworms) was provided <i>ad libitum</i>
Summer	Jan '10	Jan '10	10	Natural (daily cycles from 9.0 ± 1.6 °C to 29.6 ± 2.7 °C, photoperiod 14 L:10D)*

* C. Veloso unpublished data.

and Western, 1984). Maltase hydrolyzes the disaccharide maltose, yielding two molecules of glucose that then can be absorbed by the small intestine (Vonk and Western, 1984). Aminopeptidase-N cleaves NH_2 -terminal amino acid residues from luminal oligopeptides to produce dipeptides and amino acids absorbable by the small intestine (Ahnen et al., 1982). Small intestine sections were thawed, weighed (± 0.01 g), and homogenized for 30 s in an Ultra-Turrax® T25 homogenizer (Staufen, Germany) in 20 vol of 0.9% NaCl solution. Maltase and aminopeptidase-N activities were determined according to the method described by Vidal and Sabat (2010). Briefly, tissue homogenates (100 μL) were incubated at 25 °C with maltose or L-alanine-p-nitroanilide as substrate, and then the release rate of the products of hydrolysis were determined by spectrophotometry. We estimated the concentration of protein in the homogenate using the commercial Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL, USA). Because total protein was positively correlated with tissue mass, we only report the activity per unit of gram protein (UI g^{-1} protein = $\text{mmol min}^{-1} \text{g}^{-1}$ protein).

2.4. Statistical analysis

Differences between seasons in body size (m_b , SLV) and enzyme activity were evaluated by one-way ANOVAs. Differences in small intestine length and dry mass were evaluated by one-way ANCOVAs, using SVL or carcass dry mass as covariates. Prior to each statistical analysis, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. In some cases, data were log-transformed (e.g., small intestine dry mass) to meet the assumptions of the analyses. Values presented are means ± 1 standard error, and statistical significance was set at the 0.05 level. All the analyses were performed using the statistical package STATISTICA® (2001) version 6.0 for the Windows® operating system.

3. Results

Body mass did not differ among the four groups (Autumn: 9.1 ± 0.4 g, Winter: 8.3 ± 0.5 g, Summer: 9.4 ± 0.7 g, Experimental: 9.0 ± 0.6 g; $F_{3,37} = 0.71$, $P = 0.55$), but SVL of lizards collected during autumn was greater than those collected during summer (Autumn: 68 ± 1 mm, Winter: 66 ± 1 mm, Summer: 64 ± 1 mm, Experimental: 65 ± 1 mm; $F_{3,37} = 3.14$, $P = 0.04$). Nevertheless, beyond the statistical significance of this result, it should be noted that the difference in SVL mean values between groups was only 4 mm (6% difference).

In agreement with the hypothesis of endogenous control, small intestine length of the experimental group was similar to the winter group and both were smaller than the summer group (Fig. 2A). By contrast, and supporting an exogenous control, small intestine dry mass of the experimental group was similar to the summer group, being larger in both groups than in the winter group (Fig. 2B). Finally, specific activities of maltase and aminopeptidase-N were similar among the four groups (Fig. 3).

4. Discussion

Understanding the nature of differential effects of climate change on animal species and populations is one of the many urgent interdisciplinary challenges faced by contemporary science (Barnes et al., 2010). A premier principle in ecological physiology is that populations exposed to environmental change may crash when most individuals deteriorate, and that individuals decline when they reach a physiological state that prevents them from maintaining homeostasis, that is, a proper internal equilibrium through time.

In temperate and polar areas, the major effect of climate warming on biological systems is an increase in the length of the growing season, due to an earlier onset of spring and a postponed arrival of fall (Bradshaw and Holzapfel, 2008). Thus, if animals' physiological

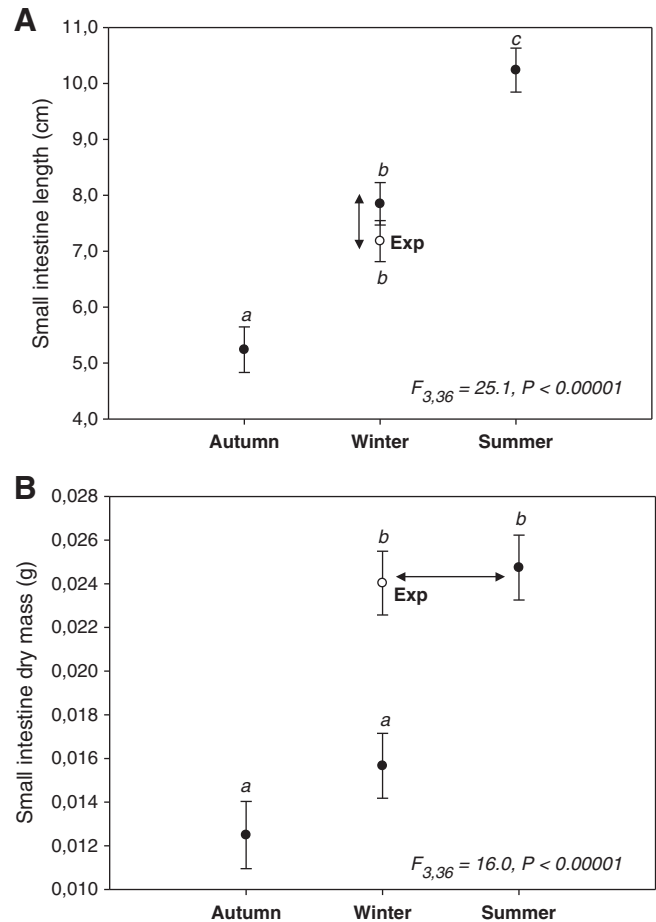


Fig. 2. Small intestine length (A) and dry mass (B) for each group. Field groups are represented by closed circles and experimental group (Exp) is represented by open circles. Values presented are least squares means ± 1 standard error. F-satistic and P-values are given inside each panel.

responses are endogenously controlled (i.e., uncoupled to an external cue), or coupled to a cue that is not affected by global warming (e.g., photoperiod), a progressive mismatch between them and their environment could be expected (Stenseth and Mysterud, 2002; Booth et al., 2006). By contrast, if physiological responses are exogenously controlled (i.e., environmentally determined), animals will be able to track the environmental change.

In this study, we observed that different digestive traits in *L. moradoensis* are under different types of control. Specifically, we found that small intestine length appears to be under endogenous control, while small intestine mass appears to be under exogenous control. Although there is no histological information for lizards about gut up- and down-regulation during the feeding and fasting cycle, information for other reptiles (Starck and Beese 2001; 2002; Lignot et al., 2005; Starck et al., 2007) and amphibians (Cramp and Franklin, 2005; Naya et al., 2009a), suggests that weight increase of the small intestine is based on loading of the enterocytes with lipid droplets and increased blood flow to the mucosa epithelium. By contrast, small intestine weight decrease is associated with the flux of lipid droplets from the enterocytes to the liver, and with the ceasing of blood flow to the gut (Starck et al., 2007). Hence, a possible explanation for our results could be that some processes related with changes in gut length, such as cell division, are mainly under endogenous control, while processes related with changes in gut mass, such as enterocytes size and content, are mainly determined by exogenous factors, such as the presence of food in the intestinal lumen. It should be noted that a

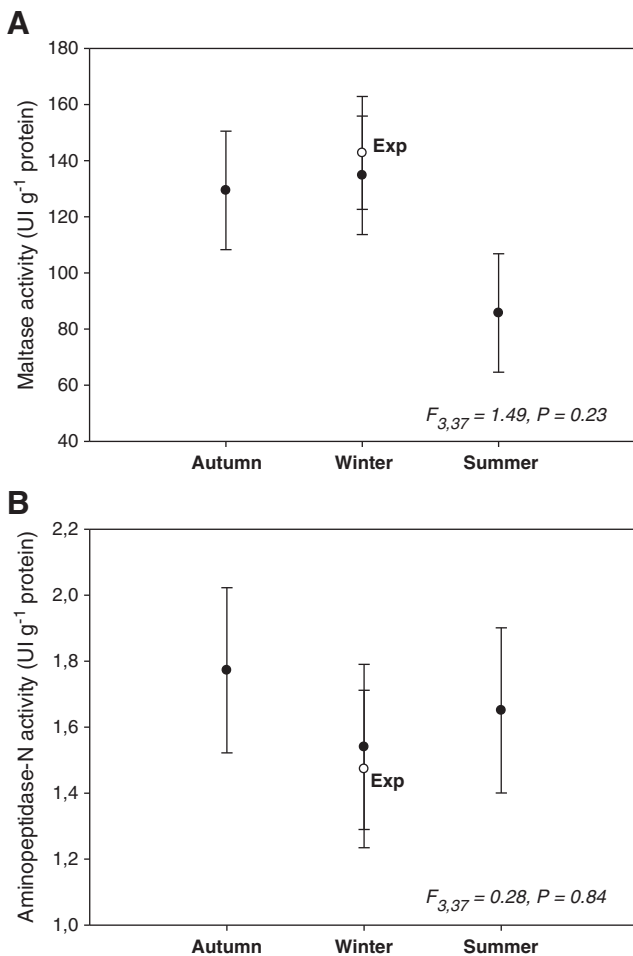


Fig. 3. Standardized activity of maltase (A) and aminopeptidase-N (B) for each group. Field groups are represented by closed circles and experimental group (Exp) is represented by open circles. Values presented are absolute means \pm 1 standard error. F-statistic and P-values are given inside each panel.

reduction in enterocyte division rate during hibernation has been reported for endotherm animals (Carey, 2005).

On the other hand, digestive enzyme activity did not show a clear pattern of seasonal variation throughout the year, nor was it affected by the experimental treatment. This result disagrees with current knowledge for other small ectotherm species inhabiting the same system—which indicates that standardized activity values are higher during summer than during winter (Naya et al., 2009a; 2009b)—but are similar to those observed for a tropical Agamid lizard (Iglesias et al., 2009) and for hibernating endotherms (Carey, 2005). In these latter animals, structure of the digestive epithelial layer remains intact during hibernation, and, when measured at the same temperature, rates of mass-specific absorption are similar to those of summer animals (Carey, 2005). Why our focal species shows a pattern of enzymatic variation closer to what was observed in hibernating endotherms and tropical lizards than in hibernating ectotherms is puzzling.

Finally, it should be noted that even in the case of small intestine dry mass, our data do not preclude the effect of a temporal combination of endogenous and exogenous control. That is, it could be possible that environmental factors acting during some time may affect physiological variables, overwriting what otherwise would be an endogenous cycle of seasonal variation. In this context, observations gathered during this and other similar experiments, indicate that lizards collected at the beginning of the hibernation period are usually depressed during the first days after capture, and then they

(i) either stay undercover if reared in natural or simulated winter conditions (regardless of food availability) or (ii) develop regular activity if reared in simulated summer conditions. Thus, it appears that it takes some time to reverse the cascade of physiological processes, related to entry into hibernation.

As was recently pointed out, phenotypic plasticity is not the only way organisms adjust to environmental change, and there are studies indicating that at least some populations are able to undergo rapid responses via selective processes (see Bradshaw and Holzapfel, 2006). Further, if environmental changes are directional and maintained in the long-term horizon, genetic changes within populations are expected to occur, resulting in different trait mean values and (or) in their plasticity. However, for several vertebrate species coping with accelerated changing conditions, and thus persistence at a given site, it appears to be closely related with the current amount of plasticity for fitness-related traits (Bertheaux et al., 2004; Charmentier et al., 2008; Gienapp et al. 2008; Telemeco et al., 2009). Accordingly, more studies aimed at determining the levels and limits of physiological flexibility will be necessary.

Supplementary materials related to this article can be found online at doi: 10.1016/j.cbpa.2011.02.005.

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