Seasonal Flexibility in Organ Size in the Andean Lizard *Liolaemus moradoensis*

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ABSTRACT The understanding of animal functioning in fluctuating environments is a major goal of physiological and evolutionary ecology. In temperate terrestrial habitats, one of the most pervasive changes in environmental conditions is that associated with the seasonal change along the year. In this study, we describe the pattern of seasonal variation in the size of nine internal organs in the lizard *Liolaemus moradoensis* from the Andes Mountains of Central Chile. We observed that the size of digestive organs was greater during summer in comparison to other seasons. Dry masses of liver and fat bodies reached maximum values during summer and minimum during spring. We suspect that lowest spring values are related with build-up costs of energetically expensive organs (e.g., digestive, muscle mass) at the end of the hibernation period. Dry mass of the heart and lungs did not show a clear pattern of variation, suggesting that cardiac and pulmonary performance were maintained throughout the year. The dry mass of kidneys was greater during winter than during summer, a result observed in other hibernating lizards but for which there is no clear explanation. Finally, the dry mass of testes showed a maximum value during autumn and a progressive reduction toward summer, indicating that reproduction occurs during autumn. When represented in a bivariate space, acquisition (digestive), distribution (heart, lungs and kidneys), storage (liver and fat bodies), and expenditure (testes) organs generate four clusters. In general terms, observed seasonal pattern of change in organ size is in agreement with those reported for other lizard species that inhabit highly fluctuating environments. J. Morphol. 271:1440–1445, 2010. © 2010 Wiley-Liss, Inc.

KEY WORDS: digestive physiology; lizards; organ size; phenotypic flexibility; seasonal changes

INTRODUCTION

Phenotypic flexibility refers to reversible changes in the traits of organisms due to changes in environmental conditions (Piersma and Drent, 2003). Nowadays, there is no doubt regarding the importance of phenotypic flexibility in many biological disciplines, such as physiology, ecology, and evolutionary biology (Pigliucci, 2001). Like the Red-Queen parabola state for species persistence (Van Valen 1973), it appears that organisms also need to continuously adjust their phenotypic traits to survive and reproduce in an ever-changing world. These responses to changing environmental conditions may include morphological, physiological, behavioral, and (or) life-history traits and can affect rates of energy acquisition and (or) rates of energy expenditure by organisms (Wiener, 1992; Bacigalupe and Bozinovic, 2002).

Some of the most important changes in an animal's environment are those associated with the change of seasons during the year. In temperate terrestrial habitats, winter poses a variety of selective pressures; the most important being the reduction in temperature that affects food availability and quality (Wunder, 1984). Accordingly, analysis of seasonal patterns of variation in organ structure and function for organisms inhabiting highly seasonal habitats is a straightforward strategy to increase our understanding on the evolutionary basis underlying organismal responses to environmental change. Indeed, understanding how and why organisms change their organs' structure and function will allow us to better appreciate their capacity for change.

The analysis of morphological variables, such as organ size, could be considered a simple, but precise, approach to the assessment of changes in tissue function. For example, studies in the digestive system have demonstrated that animals facing increased energy demands usually follow a progressive sequence of change, being the increase in

TABLE 1. *Description of the environmental conditions used to simulate winter entrance in the laboratory. Lizards did not have access to food during this period*

Initial date	Final date	T_{max} (°C)	T_{min} (°C)	Photoperiod (L:D)
April 11	April 17	25 (12 h)	10 (12 h)	12:12
April 18	May 01	20 (10 h)	8 (14 h)	9:15
May 02	May 15	15 (8 h)	6 (16 h)	6:18
May 16	May 29	10 (6 h)	4 (18 h)	3:21
May 30	June 12	6 (4 h)	2 (20 h)	0:24
June 13	Jully 01	2 (24 h)	2 (24 h)	0:24

the size of the organs the final step of this chain (Karasov and McWilliams, 2005; Naya et al., 2005). This is probably related to the fact that increases in organ size are more expensive than adjustments at other organizational levels, such as food distribution in digestive chambers or food retention time (Derting and Bogue, 1993; Nagy and Negus, 1993). Moreover, these studies also indicate that a major proportion of the overall increase in digestive function is due to adjustments in the size of digestive organs (Karasov and McWilliams, 2005).

Within this context, in this study, we tested the following hypotheses: 1) Extensive seasonal fluctuations of environmental conditions, as usually observed at high altitudes, should determine a high degree of seasonal flexibility in organ size. More specifically, we expected that individuals would have greater digestive and energy-storage organs during summer than during autumn and winter. 2) Those organs that participate in the same step of the energy (and resources) "flux chain" through the organism, e.g., acquisition, distribution, storage, expenditure, should exhibit a high temporal covariance in their size, in relation to organs that participate in other steps. More specifically, we predicted that acquisition (digestive organs), distribution and processing (heart, lungs and kidneys), storage (liver and fat bodies), and expenditure (testes) organs should group into four separate clusters, when represented in a low dimensional (bivariate) space.

To test our predictions, we used specimens of *Liolaemus moradoensis*, a medium-size lizard that inhabits the neighboring areas to "El Morado" hill in the Andes Mountains of Central Chile (Mella, 2007). In this site, lizard species are active during spring and summer (i.e., from October or November to March), with activity decreasing by mid autumn (i.e., April or May); during winter, individuals hibernate in subnivean habitats, emerging from hibernation at the beginning of the spring (i.e., late September to October).

MATERIAL AND METHODS
Specimen Collection and Morphological Determinations

Adult males of *Liolaemus moradoensis* were collected by hand from under stones and in crevices in autumn (April 2009, $n = 10$), spring (November 2009, $n = 12$), and summer (January 2010, $n = 10$) in the locality of Lagunillas (33° 36′ S, 70° 17′ W; 2,370 m above sea level) in the Andean Mountains of Central Chile. A simulated winter group ($n = 10$) was formed using additional specimens collected in April 2009 and maintained in the laboratory until July 2009 under conditions that simulate progressive winter entrance (see Table 1). We did this because during winter months, animals are in subnivean habitats and, thus, cannot be collected from the field.

Specimens were transferred to the laboratory on the day of capture, and then body mass (m_b) of each individual was measured using an electronic balance (Sartorious; ± 0.1g) and snout-to-vent length (SVL) was measured using a plastic ruler (±1 mm). Animals were reared under natural environmental conditions (without food) until the next morning, when they were cooled down by decreasing ambient temperature (0°C), and then sacrificed by decapitation. Subsequently, animals were dissected and internal organs (stomach, small intestine, large intestine, liver, kidneys, heart, lungs, abdominal fat bodies, and testes) were removed. Stomach, small and large intestines were aligned along a ruler and their lengths were measured (± 1 mm). All organs were flushed with 0.9% NaCl solution, dried together with the animal's carcass at 60°C for 1 week, and weighed (± 0.0001g; Chyo JK-180). The protocols we followed for this study conformed national and institutional guidelines for research on live animals (permit No. 4751 by the Servicio Agrícola y Ganadero).

Statistical Analysis

Differences between seasons in body size (m_b and SLV) were evaluated by one-way ANOVAs. Differences in organ lengths and dry masses were estimated separately by one-way ANCOVAs, using SVL or carcass dry mass as covariates. Before each statistical analysis, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene's tests, respectively. When necessary to meet assumptions, variables were log-transformed (e.g., stomach

TABLE 2. *Body mass (m_b), snout to vent length (SVL), and digestive organs length for each sampled season*

	Autumn ($n = 10$)	Winter ($n = 10$)	Spring ($n = 12$)	Summer ($n = 10$)	F-statistic and P-value
Body size					
m_b (g)	9.1 (0.4)	8.3 (0.5)	8.2 (0.5)	9.4 (0.7)	$F_{3,38} = 1.28$, $P = 0.29$
SVL (cm)	6.8 (0.1)	6.6 (0.1)	6.7 (0.1)	6.4 (0.1)	$F_{3,38} = 2.56$, $P = 0.07$
Organ lengths (cm)					
Stomach	2.2 (0.1)[a]	2.5 (0.1)[a]	2.6 (0.1)[a]	3.5 (0.1)[b]	$F_{3,37} = 20.9$, $P < 0.0001$
Small intestine	5.3 (0.4)[a]	7.9 (0.4)[b]	6.5 (0.3)[a,b]	10.4 (0.4)[c]	$F_{3,37} = 32.4$, $P < 0.0001$
Large intestine	1.8 (0.1)[a]	1.9 (0.1)[a]	1.9 (0.1)[a]	2.5 (0.1)[b]	$F_{3,37} = 11.9$, $P < 0.0001$

Values presented are absolute means (± 1 standard error) for m_b and SVL, and least squares adjusted mean (± 1 standar error) for digestive organ lengths. Different letters indicate significant differences between means.
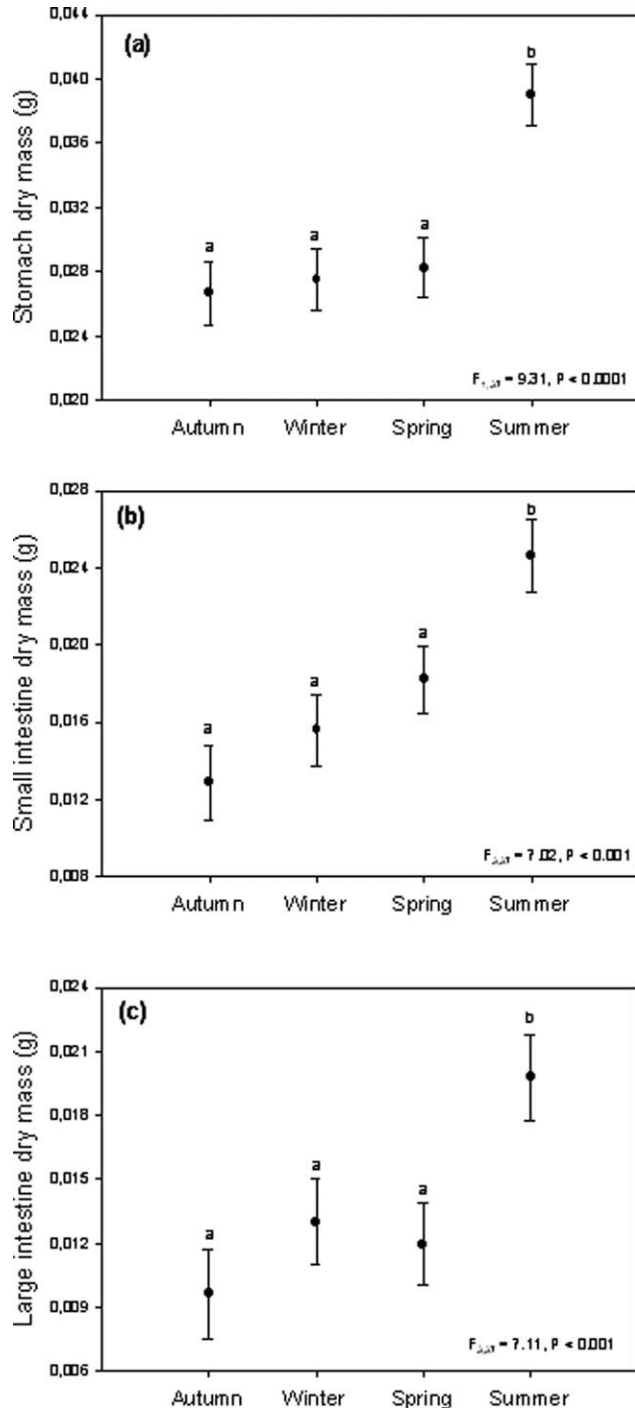
Fig. 1. Dry masses of stomach (**a**), small intestine (**b**), and large intestine (**c**) for each season. Values presented are least squares means ± 1 standard error. *F*-statistic and *P*-values are given inside each panel. Different letters indicate significant differences between means.

length, large intestine and liver dry masses). To evaluate the pattern of covariation among organs, we conducted a principal component analysis (PCA), using as the raw data the residuals of the relationship between each organ dry mass and carcass dry mass (to remove the effect of differences in body size). This multivariate technique reduces the number of correlated variables to a smaller number of uncorrelated variables that usually

have a more intuitive interpretation (Manly, 1986). Statistical significance was established at the 0.05 level. All the analyses were performed using STATISTICA, version 6.0.

RESULTS

Body mass and SVL did not differ between seasons (Table 2). Lengths and dry masses of all digestive organs were greater in summer than in the other seasons (Table 2, Fig. 1). Dry masses of liver and fat bodies reached maximum values during summer and minimum values during spring (Fig. 2a,b). Dry mass of the heart and lungs did not show a clear pattern of variation along the year (Fig. 2c,d), whereas the dry mass of kidneys was lower during summer than during the other seasons (Fig. 2e). Finally, dry mass of the testes showed a progressive reduction from autumn to summer (Fig. 2f).

Regarding to the pattern of covariation among organs masses, the first two components of the PCA explained 38.8 % and 20.5%, respectively, of the nonsize related variance. In addition, when organs are represented in the bivariate space generated by these two components, it can be observed that they are clearly clustered according to their function (Fig. 3; Table 3). Specifically, acquisition (digestive) and storage (liver and fat bodies) organs are separated from distribution (heart, lungs and kidneys) and expenditure (testes) organs in the first component, whereas acquisition and distribution organs are separated from storage and expenditure organs in the second component.

DISCUSSION

Understanding of animal functioning in fluctuating environments is one of the major goals of physiological and evolutionary ecology (Karasov and Martínez del Rio, 2007). As pointed out earlier, the analysis of seasonal variation in organs size could be considered a first approach toward this main goal.

The digestive system of vertebrate animals has been demonstrated to be one of the most flexible systems in relation to changes in environmental conditions (for recent review see Starck, 1999, 2005; McWilliams and Karasov, 2001; Naya and Bozinovic, 2004; Naya et al., 2007). In this sense, seasonal changes in digestive attributes have been reported for several vertebrate species, including amphibians (Juszczyk et al., 1966; Naya et al., 2009a), reptiles (Latif et al., 1967; Starck et al., 2007), mammals (Myrcha, 1964; Derting and Hornung, 2003), and birds (Davis, 1961; Maldonado et al., 2009). In agreement with these studies, we found that digestive organs of *L. moradoensis* were larger during the warmer and more productive months of the year, which suggest that foraging activity in this species occurs during spring and summer. However, and in contrast to our first expectation, we found an abrupt increase in digestive organ mass from spring to
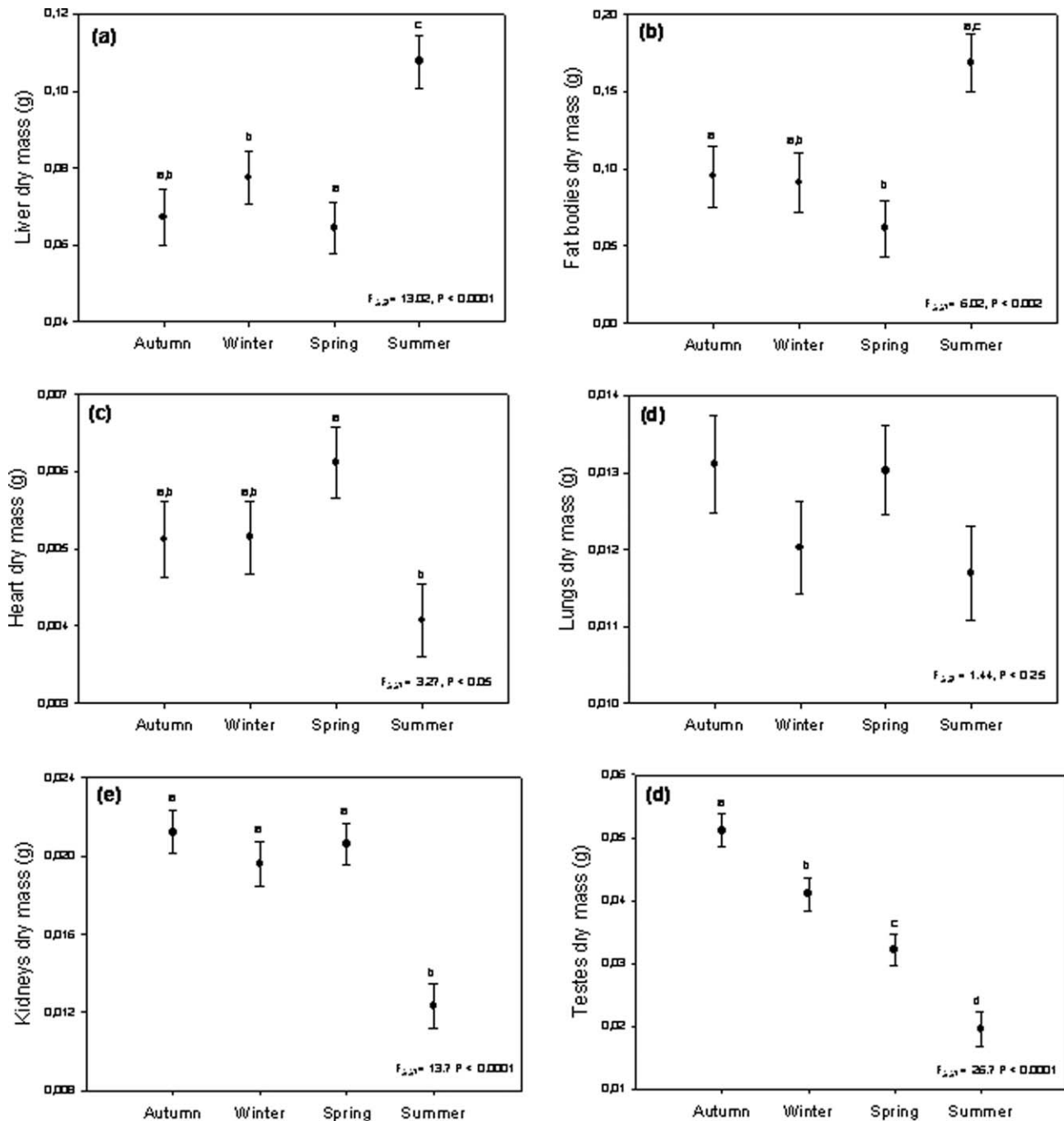
Fig. 2. Dry masses of liver (**a**), abdominal fat bodies (**b**), heart (**c**), lungs (**d**), kidneys (**e**), and testes (**f**) for each season. Values presented are least squares means $\pm$ 1 standard error. F-statistic and P-values are given inside each panel. Different letters indicate significant differences between means.

summer, instead of a more progressive rise from hibernation (i.e., autumn or winter) to the peak of foraging activity (i.e., summer). Although we have no clear explanation for this result, we suspect that it may be related with winter strength and duration for the particular year evaluated. Field observations indicated that snow cover melting and plant phenology was delayed for 1 or 2 months with respect to other years (C. Veloso personal observation), and

thus, it could be possible that animals arose from hibernation (and reasume feeding activity) later during the year that we found them in the field.

Studies on the seasonal pattern of variation in liver and fat bodies for temperate lizards are common (e.g., Dessauer, 1955; Derickson, 1976; Heideman, 1994; Holmes and Cree, 2006). Most indicate that lipid storage occurs during late summer or early autumn, and then, these reserves are used
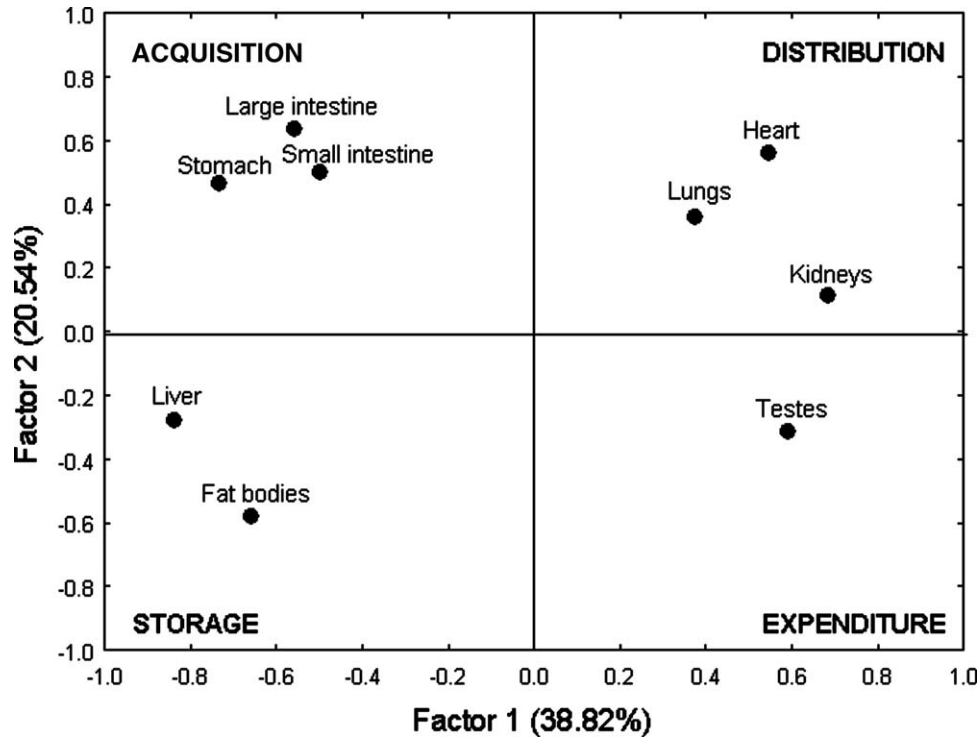
Fig. 3. Graphical representation of the principal component analysis (PCA) for organs dry masses. Total amount of variance explained by each component is given in the graph axes.

during winter and spring for maintenance, gamete production, and other reproductive activities (e.g., Guillette and Casas, 1981; Selcer, 1987; Mendez et al., 1988). Accordingly, here we found for *L. moradoensis* that both organs reached a maximum size during summer and minimum during spring season. Nevertheless, given the pattern of variation for reproductive organs (see below), we hypothesize that lowest spring values are more related with the maintenance cost during hibernation. In this sense, we suspect that spring captured animals had only just awoken from dormancy and had not yet have the time to resupply their reserves.

On the other hand, we did not find a clear seasonal trend for the case of heart and lung masses, which supports the idea that cardiac and pulmonary performance is maintained during dormancy (Secor, 2005). Interestingly, the dry mass of kidneys was noticeably greater during winter than during summer time, a result that is congruent with data for other lizard species and for which there is no clear explanation (see Naya et al., 2009b). Finally, the dry mass of testes reached a maximum value during autumn and then continuously decreased until summer. This suggests that gamete production occurred in early autumn and that mating started shortly thereafter, as is the case of other Mexican lizards (Ramirez et al., 1998) and at least one South American *Liolaemus* species (Ramirez, 1991).

The "barrel model" is a clever analogy that illustrates energy fluxes through an animal (Wiener, 1992). In this model, an organism is represented by a barrel, with input energy constraints, e.g., foraging, digestion and absorption, symbolized by funnels connected in tandem, and energy outputs, e.g., maintenance, growth, reproduction, symbolized by a series of spouts arranged in parallel. When environmental conditions change, organisms are able to respond, by adjusting the size of the funnels, the output flow through the spouts, or the fluid stored inside the barrel. The barrel analogy had an influential role in physiological ecology research during the 1990s, mainly because of its conceptual simplicity. However, despite its conceptual simplicity, very few attempts have been made to test the general predictions arising from this model. One of these predictions is that organs that

TABLE 3. *Relative weights for each independent variable on the first two components of the PCA*

	PC 1	PC 2
Stomach	−0.735	0.467
Small intestine	−0.498	0.500
Large intestine	−0.561	0.641
Liver	−0.840	−0.275
Fat bodies	−0.660	−0.580
Heart	0.544	0.561
Lungs	0.375	0.364
Kidneys	0.682	0.116
Testes	0.590	−0.311

participate in the same step in the energy "flux chain" should exhibit higher temporal covariance in their size, in relation to organs that participate in other steps. In other words, organs performing similar functions should change in much the same magnitude and direction througout the year relative to organs performing different funcitons. In agreement with this prediction, we found that acquisition (stomach, small and large intestine), distribution (heart, lungs, and kidneys), storage (liver and fat bodies), and expenditure (testes) organs of *L. moradoensis* generate four separate clusters. This suggests that the complexity of the processes by which organisms obtain, store, and expend energy and resources can be reduced when analyses are done at certain organizational levels (e.g., morphometry) and temporal scales (e.g., season).

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