Frog somatic indices: Importance of considering allometric scaling, relation with body condition and seasonal variation in the frog *Leptodactylus latrans*

Julie Céline Brodeur[a,d,*], Josefina Vera Candioti[b,d], Maria Jimena Damonte[a], Maria Florencia Bahl[c,d], Maria Belen Poliserpi[a], Maria Florencia D'Andrea[a,d]

[a] *Instituto de Recursos Biológicos, Centro de Investigaciones de Recursos Naturales (CIRN), Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Buenos Aires, Argentina*
[b] *Agencia de Extensión Rural Venado Tuerto, Instituto Nacional de Tecnología Agropecuaria (INTA), Venado Tuerto, Santa Fe, Argentina*
[c] *Centro de Investigaciones del Medioambiente (CIM), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina*
[d] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

A R T I C L E   I N F O

A B S T R A C T

Snout-vent length (SVL) and liver, gonad, fat bodies and carcass weight data from 661 individual *Leptodactylus latrans* frogs collected over ten years in the Pampa Region of Argentina were analyzed to evaluate the best approach for expressing the corresponding somatic indices. The seasonal variation of these indices and their respective correlation with body condition was also examined. Results obtained demonstrated that the weight of all examined tissues and organs vary in an allometric manner in function of SVL, which implies that scaled somatic indices should be employed in this species. The study also highlights the fact that size-independent somatic indices are more easily obtained if the scaling exponent is defined through a non-linear regression of mass on length rather than by performing a standardized major axis regression of lnweight on lnlength. In the case of liver, fat and carcass, the non-linear regression curves were not statistically different amongst sexes and so a single relationship was described for both males and females *L. latrans*. Logically, the relationships between SVL and male and female gonad weight varied on distinct scale, and so it was necessary to analyze ovaries and testis separately. Scaling factors equal to 5.03, 3.11 and 2.75 were calculated to respectively estimate fat (SFI), liver (SLI) and carcass (SCI) scaled indices of *L. latrans*. In the case of the scaled gonadal index (SGI), scaling factors equal to 3.81 and 6.49 were used to calculate male and female indices. In both sexes, the seasonal variation in SFI and SGI was perfectly opposite, SGI being at its maximum in the spring when SFI was near zero, and reaching its lowest values in February-March when SFI increased. The amplitude of these changes was, nevertheless greater in females, representing a 4–5 times order of variation, in contrast to a 2–3 times order of change in males. In both sexes, SLI exhibited a 30% drop from October to December, although this loss was completely recovered in the second half of the summer (December to March). SLI was the somatic index that best correlated with the 14–18% natural variation in body condition that was observed over the spring-summer season. Nevertheless, carcass-related energy reserves were also of significant importance for frog metabolism as SCI varied very closely with body condition, explaining 75–80% of is variation. Results obtained illustrate the fact that no single somatic index can solely illustrate body condition because of the intricate relationship existing between SGI and SFI, and the importance of carcass-related energy reserves. In view of all the above, body condition comes out as the ideal monitoring endpoint for acquiring information on frog energy status.

1. Introduction

The world is currently experiencing a biodiversity crisis (Pimm et al., 2014; Williams et al., 2015) and amphibians are the most rapidly declining group of vertebrates (Stuart et al., 2004; Pounds et al., 2006; Roelants et al., 2007; IPBES, 2019). It is therefore essential to develop, extend and improve amphibian monitoring programs so as to gather essential information on the status and health of amphibian populations

worldwide. In this sense, biomonitoring studies constitute a valuable assessment tool, as they rely on biomonitor species to understand the temporal and spatial variation of environmental quality and its effects on wildlife (Huggett et al., 2018). Biomonitor species are organisms that provide quantitative information on habitat quality through a variety of somatic, physiological or biochemical measures. The somatic measures most frequently employed in biomonitoring studies include body condition and diverse somatic indices such as the hepatosomatic index or the gonadosomatic index. These so-called somatic or condition indices are usually simple measures of mass (of the entire organism or specific organs) in terms of total length or other morphometric measure of the animal (tarsus length, girth, etc.) (MacCracken and Stebbings, 2012). The ultimate goal of these condition indices is to use the variations in mass for a given body size as a surrogate for the variation in the size of the energy reserves and the individual's general well-being (Peig and Green, 2010).

To be useful, a condition index must accurately reflect energy stores and be independent of body size so that animals of different size can be adequately compared (MacCracken and Stebbings, 2012). Over the last decades, it has become clear that simple ratio-based condition indices are dependent and correlated with body size (Jakob et al.; 1996). This is because they do not properly take into account the allometric scaling of growth: i.e. the fact that body growth involves not only a variation in body size but also a variation of body composition and of the proportional mass of energy stores (Peig and Green, 2009, 2010). To resolve this, Peig and Green (2009) introduced a new body condition factor named the scaled mass index (SMI). The SMI is based on the fact that total body mass is related to body length by the power function Mass = a Length^b; a and b being constants (Hoppeler and Weibel, 2005). The SMI method consists in quantifying the scaling exponent b for the specific species and weight – length relationship examined, and then calculating the predicted body mass of studied individuals at a determined body length (Peig and Green, 2009). Peig and Green (2009, 2010) recommend estimating b through a standardized major axis (SMA) regression of lnMass on lnLength. They call bSMA the scaling exponent obtained in this way. However, in a recent study on the frog *Leptodactylus latrans*, our group demonstrated that a truly size-independent SMI value is better obtained by defining the scaling exponent through a non-linear regression of mass on length rather than by performing a standardized major axis regression of lnweight on lnlength (Brodeur et al., 2020).

The SMI gradually gained popularity in various disciplines as a body condition index, and has now been successfully employed in studies with fish (Maceda-Veiga et al., 2014; Brodeur et al., 2017; Dalzochio et al., 2018; Wuenschel et al., 2019), birds (Hudin et al., 2016; Nip et al., 2018; Fanny-Linn et al., 2019; English et al., 2018), mammals (Tête et al., 2013; Rodríguez-Estival and Smits, 2016; Abolins et al., 2018; Risco et al., 2018), and amphibians (MacCracken and Stebbings, 2012; Sánchez et al., 2013; Alvarado-Rybak et al., 2018; Romano et al., 2018; Brodeur et al., 2020). However, although the necessity to consider the allometric scaling of growth is widely recognized when calculating the whole body condition index, many researchers still disregard the fact that allometric growth also occurs at the organ level. Indeed, most studies on amphibians and other vertebrates still use a percentage ratio index in which organ weight is divided by body weight and then multiplied by 100, to express somatic indices such as the hepatosomatic index or the gonadosomatic index (Edge et al., 2013, Li et al., 2018; Regnault et al., 2018, Paunescu et al., 2018; Du et al., 2019). Sometimes, even if body condition index is expressed as SMI (Wuenschel et al., 2019). Only a few pioneering studies with rodents, have demonstrated the scaled nature of organ growth and expressed somatic indices as scaled indices, according to the calculation of SMI (Tête et al., 2013; Rodríguez-Estival and Smits, 2016).

In this context, it is crucial to clearly determine the correct manner of informing somatic indices in frogs so as to design efficient and informative amphibian monitoring programs. In parallel, it is also

necessary to have a clear understanding of the tissue-specific location and seasonal variations of energy accumulation in frogs, to identify the most informative somatic indices and to determine how these should be calculated. Anurans can store energy in the form of fat or glycogen (Wells, 2007). Major energy storage sites include the liver, skeletal muscles, female gonadal tissue, finger-like fat bodies located at the anterior end of the gonads, and cutaneous and subcutaneous fat bodies (carcass fat) (Fitzpatrick, 1976; Wygoda et al., 1987, Fournier and Guderley, 1993; Wells et al., 1995, Lu et al., 2008). These energy stores are used for metabolic maintenance, egg production, coping with food shortage, sustaining life over the winter, and for breeding activities in the spring (Pider et al., 1992; Lu et al., 2008). Because of their low maintenance energy requirements, amphibians can rapidly gain energy reserves during specific months and use it at another time of the year. They can also readily shift allocation of energy reserves between reproduction and growth according to seasonal demands and food availability (Wells, 2007; Lardner and Loman 2003). As a consequence, marked seasonal changes occur in liver and muscle glycogen and body lipids of amphibians living in seasonal environments (Mizell, 1965).

To further advance amphibian monitoring strategies, the objectives of the present study were to: 1) examine the shape of the relationships existing between total body weight and the weight of different organs and tissues of the South American common frog, *L. latrans*, to determine if the different somatic indices should be expressed in terms of ratios, in the case of linear relationships, or in terms of a scaled mass index, in the case of power functions, 2) determine which somatic indices are more closely related with body condition, and 3) examine the seasonal variations exhibited by the various somatic indices. *L. latrans* was selected as the test species because it was previously suggested as a potential biomonitor of the impacts of intensive agricultural production on the South American Pampean herpetofauna (Brodeur et al., 2011; Brodeur and Vera Candioti, 2017), and a recent study by our group previously defined how to best calculate body condition in this species (Brodeur et al., 2020).

2. Methods

2.1. Studied species

The semi-aquatic frog *Leptodactylus latrans* was selected as the test species for the current study. *L. latrans* is a large long-legged anuran species that can reach up to 120–140 mm in snout-vent length (SVL) (Cei, 1980). It is widely distributed over South America, east of the Andes, from Venezuela to Argentina, and it is one of the few amphibian species in which males are larger than females (Gallardo, 1987; Brodeur et al., 2020). Mature males typically have large well developed arms and two conical spines on the first finger of the hand. *L. latrans* occurs in a wide range of habitats, including wet grasslands, riverbanks, tropical forests and highly modified agroecosystems (Heyer et al., 2010). It is a generalist consumer whose diet consists of beetles, arachnids, lepidoptera, ants, annelids and other smaller anurans (Lajmanovich, 1996; Maneyro et al., 2004). In both sexes, growth is faster from metamorphosis to about three years-old, and decreases afterward (López et al., 2017). Longevity is five years for both males and females and sexual maturity is reached during the first year, when attaining a minimal size of 60 mm or a body mass of around 33 g (López et al., 2017). Reproductive activity and calling are typically observed after abundant rainfalls in the spring and summer. The conservation status of *L. latrans* is listed as "Least Concern" in view of its wide distribution, tolerance to a broad range of habitats, and presumed large population (Heyer et al., 2010).

2.2. Frog samplings

L. latrans morphometric data used in the present study are a compilation of data collected over ten years (from 2006 to 2016) in six

geographically distinct locations of the Pampa Region of Argentina. The six locations consisted of agricultural lands, pasture fields or natural reserves situated within the localities of 1) Magdalena, 2) Chivilcoy, 3) Pergamino, 4) Otamendi, in Buenos Aires Province, 5) Venado Tuerto in Santa Fe Province, and 6) Cerrito and Crespo in Entre Rios Province. Overall, data from a total of 661 adult and juvenile frogs were considered in the present study. In all cases, frogs were captured by hand at sunset during spring and summer months of the southern hemisphere (from October to March). Captured frogs were placed in plastic containers fitted with air holes and containing river or pond water to a depth of approximately 5 cm. On the next morning, frogs were anaesthetized individually in water containing 100 mg/L of tricaine methanesulphonate and sacrificed by cutting the neural cord behind the brain. Snout-vent length was measured to the nearest 0.01 mm with a digital Vernier caliper and body weight was measured to the nearest 0.01 g with an electronic balance. A longitudinal cut was made in the abdomen, and liver, gonads and fat bodies were excised carefully, blotted dry and weighed to the nearest 0.001 g with an electronic balance. Animal captures in Buenos Aires Province were realized under permits from "Dirección de Administración de Areas Protegidas y Conservación de la Biodiversidad" and from the National Parks administration of Argentina (for the Natural Reserve Otamendi). Frog captures in Magdalena, were realized in the Natural Reserve "El Destino" under a permit from "Dirección de Áreas Naturales Protegidas" of the "Organismo Provincial para el Desarrollo Sostenible" (OPDS) of Buenos Aires Province, Argentina.

2.3. Body condition and somatic indices

Body condition was computed through the scaled mass index (SMI) method as defined by Peig and Green (2009). The SMI method consists in calculating the predicted body mass of studied individuals at a given SVL using the function Mass = a Length ^{b}, where b is species-specific. In the present study, a value of 3.11 was assigned to the exponent b, as previously determined *for L. latrans* (Brodeur et al., 2020). Body condition was calculated for the SVL corresponding to the average SVL of all sampled frogs.

After examining the shape of the relationship existing between the snout-vent length of the frogs and the weight of their liver, gonads, fat bodies and carcass; hepatic, gonadal, body fat and carcass somatic indices were calculated as scaled indices, through the SMI method. Gonadal indices were examined considering frogs from all maturity stages to obtain an overall view of the variability of the relationship existing between gonad weight and body weight. Carcass weight was calculated by subtracting the weight of the liver, the fat bodies, the gonads and the digestive tract from total body weight. The carcass index was calculated in the present study because the skeletal muscle, which accumulates glycogen, and cutaneous and subcutaneous fat bodies (carcass fat) may be a significant site of energy accumulation in amphibians. For each tissue/organ examined, the exponent b was estimated in two different ways to identify the most efficient approach: 1) it was directly defined through a non-linear power function regression, and 2) it was determined through a standardized major axis (SMA) regression of lnMass on lnLength (the so called "bSMA" as defined by Peig and Green (2009, 2010). In all cases, scaled indices were calculated for the SVL corresponding to the average SVL of all sampled frogs. Calculated indices were respectively named scaled liver index (SLI), scaled gonad index (SGI), scaled fat index (SFI), and scaled carcass index (SCI).

2.4. Seasonal variation of the somatic indices

To examine and compare the seasonal variation of both body condition and the different somatic indices computed, data calculated on different months in a single population of frogs were plotted and statistically analyzed (more details below). Data used for this part of the work were those collected from *L. latrans* sampled in the Natural Reserve "El Destino", Magdalena, Buenos Aires Province, Argentina. Animals from this site were selected because they were the most frequently sampled and also the less likely to be impacted by agriculture or other human activities. "El Destino" Reserve was created in 1979 and extends over 18.54 km^2. It is, furthermore, situated within the "Parque Costero del Sur" a UNESCO Biosphere Reserve of 265 km^2 created in 1997. Within the reserve, frogs were captured in inundated pastures and along the margins of Primera Estancia River. Data gathered in October 2016, November 2009, December 2011, February 2010 and March 2010 were used in the analysis. It is important to recall here, that samplings occurred in the Southern Hemisphere and so, October and November correspond to springtime, December is early summer and February-March correspond to late summer.

2.5. Data analysis

The presence of statistically significant differences amongst the scaling exponents calculated for different power functions was determined through Akaike's Information Criteria (AICc) provided in the "Compare" function of the non-linear regression fitting module of GraphPad Prism Version 5.03 software. The generalized linear models (GLM) function of Systat 11 software package was used to compare the slopes of two linear regressions. This was realized by testing for the existence of an interaction between the categorical variable and the covariate. Because normality and equal variance of the data could not be achieved, body condition and somatic indices calculated in different months were compared using a Kruskall-Wallis non-parametric one-way analysis of variance on ranks followed by Dunn's multiple comparison procedure. All linear regressions, non-linear power function regressions, correlations and analysis of variance were realized using the SigmaStat statistical software included in the SigmaPlot version 12.5 software (SPSS, Chicago, IL, USA). The criterion for significance was set at $p < 0.05$ in all cases.

3. Result

Body weights of examined frogs ranged between 3.63 and 153.14 g, with an average of 38.62 ± 0.85 g (mean ± S.E.). For its part, SVL ranged between 60 and 105 mm, with an average of 72.45 ± 0.50 mm (mean ± S.E.).

3.1. Body fat index

Only frogs exhibiting a maximum or near maximum mass of fat for their length were considered in order to characterize the relationship between body fat and SVL. Fat varied in an allometric manner in function of SVL in both males and females *L. latrans* (Fig. 1a). As the non-linear regression curves were not statistically different amongst sexes, a single relationship between fat and SVL was described by the power function: $Y = X^{5.03}$ (95% CI of the scaling exponent: 4.31–5.75) (Fig. 1a). In contrast, a scaling exponent of 8.51 was estimated when indirectly calculating a bSMA exponent (i.e. dividing the slope of an OLS regression of Ln-transformed fat and SVL data by the correlation coefficient). SFI calculated using the bSMA scaling factor (8.51) presented a significant inverse relationship with SVL (Pearson correlation coefficient = −0.409, p < 0.0001), indicating that bSMA-calculated scaled fat is not completely independent from structural size. In contrast, SFI calculated with the scaling exponent estimated from the non-linear power function regression (5.03) was independent from SVL (Pearson correlation coefficient = −0.019, p = 0.713). Based on these results, the use of a scaling factor of 5.03 was considered optimal for estimating SFI in *L. latrans,* and was used in further analysis. The SFI correlated slightly better with body condition in males than in females (Table 1), and the slope of the linear regression curve calculated between SFI and body condition were not significantly different amongst
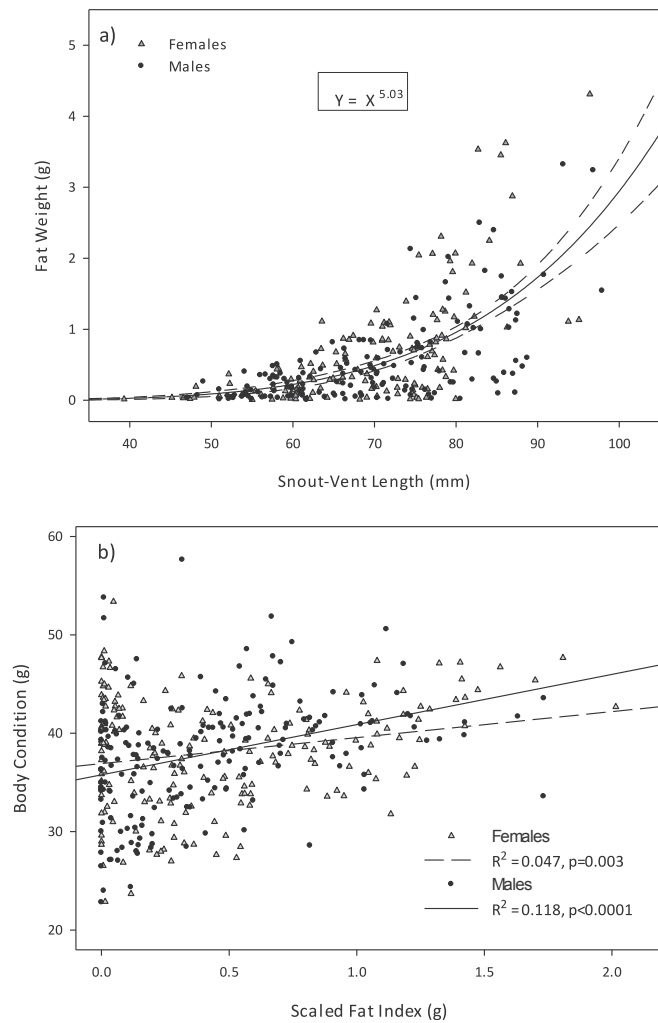
Fig. 1. a) Relationship between fat weight and snout vent-length of *L. latrans*. The mathematical equation describing the regression line (full line) is indicated in the box. Dashed lines represent the 95% confidence interval of the regression line. b) Relationship between scaled fat index and body condition of males and females *L. latrans*. Linear regression curves obtained for each sex are illustrated. Slopes were not significantly different amongst sexes (p = 0.069).

Table 1
Pearson correlation coefficients and significance values describing the relationship between scaled organs/tissues indices and body condition in *L. latrans*.

| | Body condition | | |
	All	Males	Females
Scaled fat index	0.260	0.343	0.217
	P < 0.0001	P < 0.0001	P = 0.003
Scaled liver index	0.688	0.716	0.668
	P < 0.0001	P < 0.0001	P < 0.0001
Scaled gonad index	0.248	0.254	0.378
	P < 0.0001	P < 0.0001	P < 0.0001
Scaled carcass index	0.956	0.961	0.947
	P < 0.0001	P < 0.0001	P < 0.0001

sexes (Fig. 1b). Overall, the SFI explained relatively little of the inherent variability of body condition: 4.7% in females and 11.8% in males (Fig. 1b).

3.2. Hepatic index

Liver weight varied in an allometric manner in function of SVL in
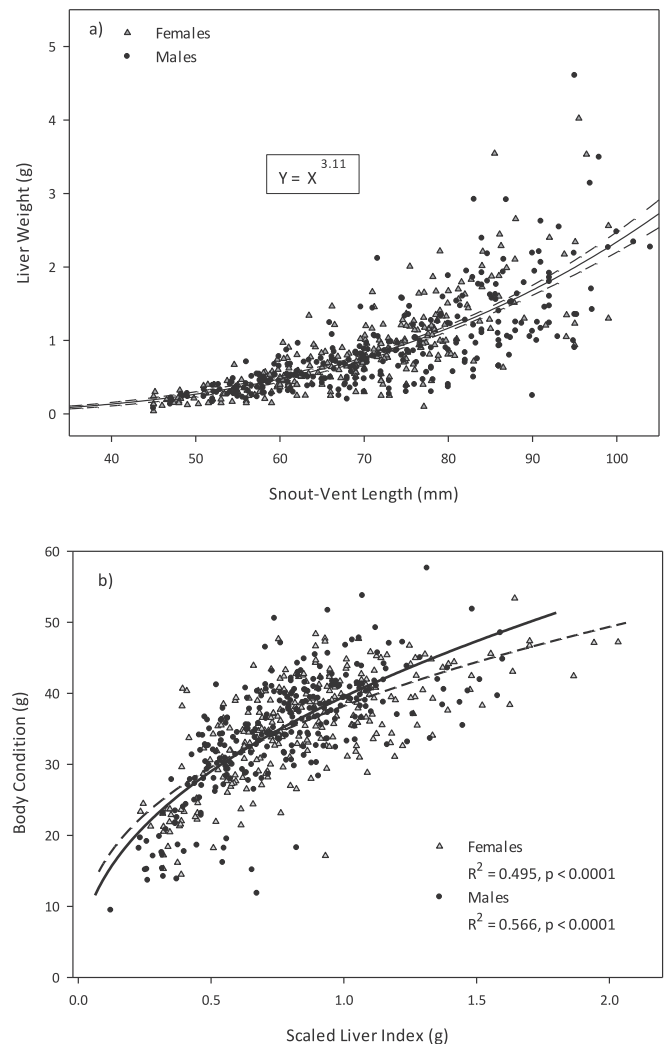

Fig. 2. a) Relationship between liver weight and snout vent-length of *L. latrans*. The mathematical equation describing the regression line (full line) is indicated in the box. Dashed lines represent the 95% confidence interval of the regression line. b) Relationship between scaled liver index and body condition of males and females *L. latrans*. Non-linear regression curves obtained for each sex are illustrated. Scaling exponent were significantly different amongst sexes (P < 0.05).

both males and females *L. latrans* (Fig. 2a). As the non-linear regression curves were not statistically different amongst sexes, a single relationship was described between liver weight and SVL by the power function: $Y = X^{3.11}$ (95% CI of the scaling exponent: 2.87–3.36). The indirectly calculated bSMA exponent equaled 3.94. As was the case for the SFI, SLI calculated with the bSMA exponent exhibited a significantly inverse relationship with SVL (Pearson correlation coefficient = −0.346, p < 0.0001). Again, this dependence on SVL disappeared when the SLI was calculated using the exponent derived from the non-linear power regression (i.e. 3.11). (Pearson correlation coefficient = 0.011, p = 0.080). The use of a scaling factor of 3.11 is thus recommended and was employed in the present study to estimate SLI in *L. latrans*. Body condition correlated much better with SLI than with SFI (Table 1). Although a significant linear relationship could be fitted between SLI and body condition in both sexes (data not shown), a slightly better adjustment was obtained when fitting a power curve (Fig. 2b). The scaling exponents of the power curves were significantly different between males and females. The SLI explained respectively 56.7% and 49.5% of the variance of body condition in males and females, respectively (Fig. 2b).
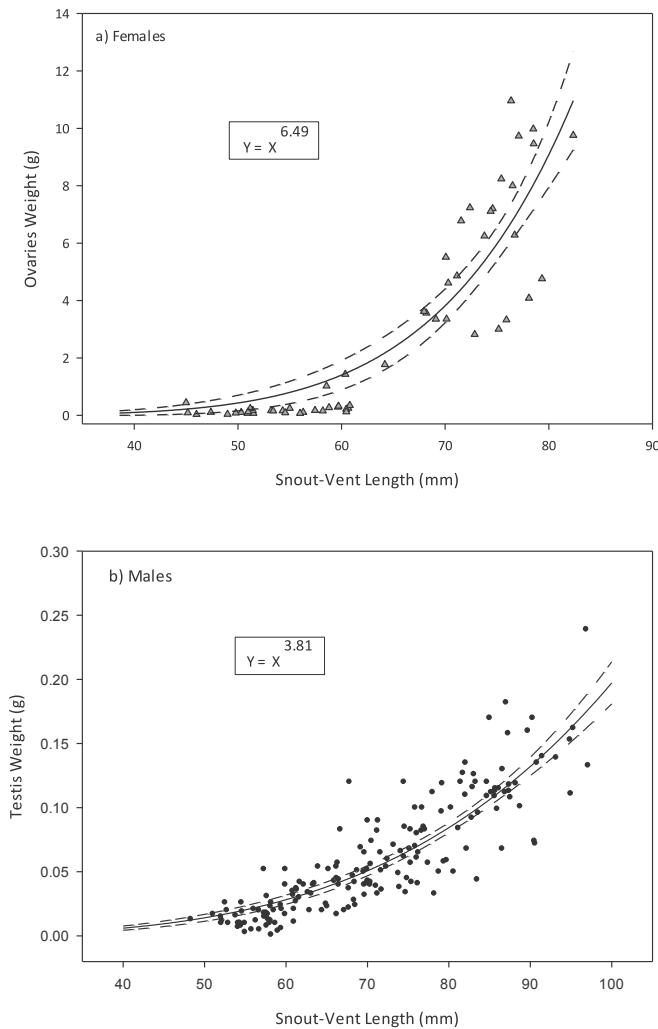
Fig. 3. Relationship between a) ovaries weight and b) testis weight and snout vent-length in *L. latrans*. The mathematical equations describing the regression line (full line) are indicated in boxes. Dashed lines represent the 95% confidence interval of the regression lines.

3.3. Gonadal index

As could be expected, it was necessary to analyze male and female gonadal indices separately as they varied on distinct scales (Fig. 3). Still, both male and female gonad weight presented an allometric relationship with SVL, which was described by the power function: $Y = X^{3.81}$ in males (95% CI of scaling exponent 3.81: 3.44–4.18) and $Y = X^{6.49}$ in females (95% CI of scaling exponent 6.49: 4.98–7.99). The indirectly calculated bSMA exponents equaled 5.50 for males and 10.66 for females. As was the case for both somatic indices above described, SGI calculated with the bSMA exponent exhibited a significant inverse relationship with SVL in both males (Pearson correlation coefficient = −0.280, p < 0.0001), and females (Pearson correlation coefficient = −0.403, p < 0.0001). As occurred with the other body indices, the dependence of SGI on SVL disappeared when this index was calculated using the exponent derived from the non-linear power regression (i.e. 3.81 for males and 6.49 for females). (Males Pearson correlation coefficient = −0.066, p = 0.296; Females Pearson correlation coefficient = 0.035, p = 0.582). The use of scaling factors equal to 3.81 and 6.49 is thus recommended to calculate males and females *L. latrans* SGI and were employed in the current study. SGI correlated slightly better with body condition in females than in males, the two sexes presenting correlation coefficients of 0.254 and 0.378,
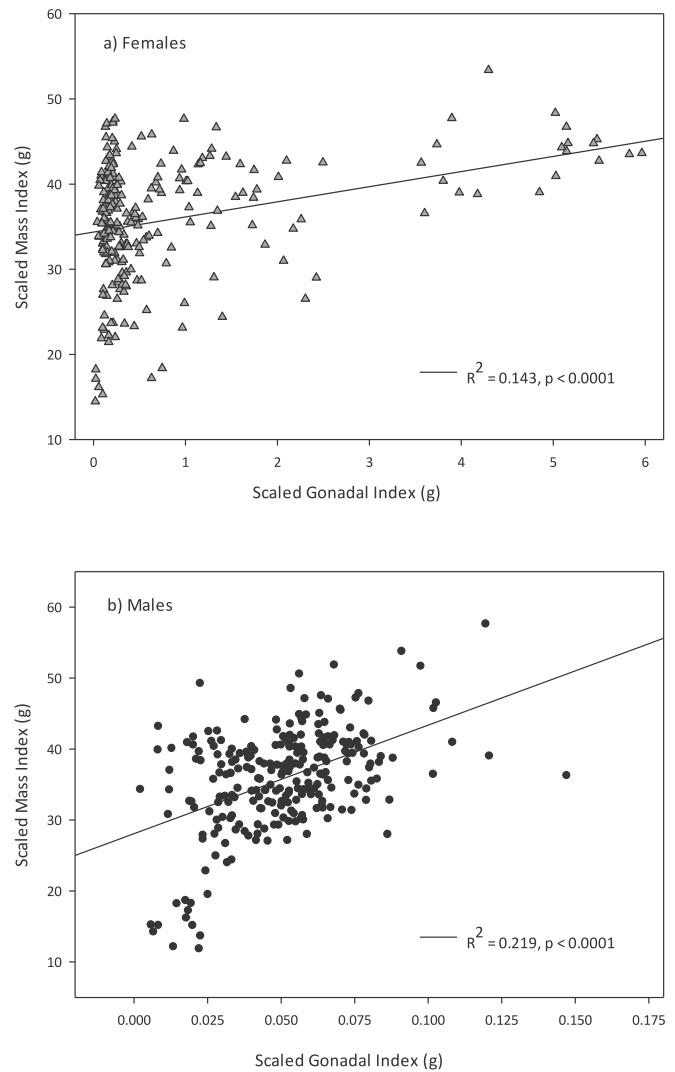

Fig. 4. Relationship between scaled gonadal index and scaled mass index of a) females and b) males *L. latrans*. The linear regression curve obtained for each sex is illustrated. Slopes were significantly different amongst sexes (p < 0.05).

respectively (Table 1). Slope of the linear regression curve calculated between SCI and body condition was significantly different amongst sexes (Fig. 4). Overall, SGI explained little of the variance inherent to the SMI: 14.3% in females and 21.9% in males (Fig. 4).

3.4. Carcass index

Carcass weight varied in an allometric manner in function of SVL in both males and females *L. latrans* (Fig. 5a). As the non-linear regression curves were not statistically different amongst sexes, a single relationship between liver weight and SVL was described by the power function: $Y = X^{2.75}$ (95% CI of the scaling exponent: 2.61–2.88). The indirectly calculated bSMA exponent equaled 3.06. As was the case for all somatic indices above described, SCI calculated with the bSMA exponent exhibited a significantly inverse relationship with SVL (Pearson correlation coefficient = −0.158, p = 0.0003). Again, this dependence on SVL disappeared when the SLI was calculated using the exponent derived from the non-linear power regression (i.e. 2.75). (Pearson correlation coefficient = 0.087, p = 0.052). The use of a scaling factor of 2.75 is thus recommended and was employed in the present study to estimate SCI in *L. latrans*. SCI correlated strongly with body condition in males and females, the two sexes presenting correlation coefficients of 0.961 and 0.947, respectively (Table 1). Slope of the linear regression
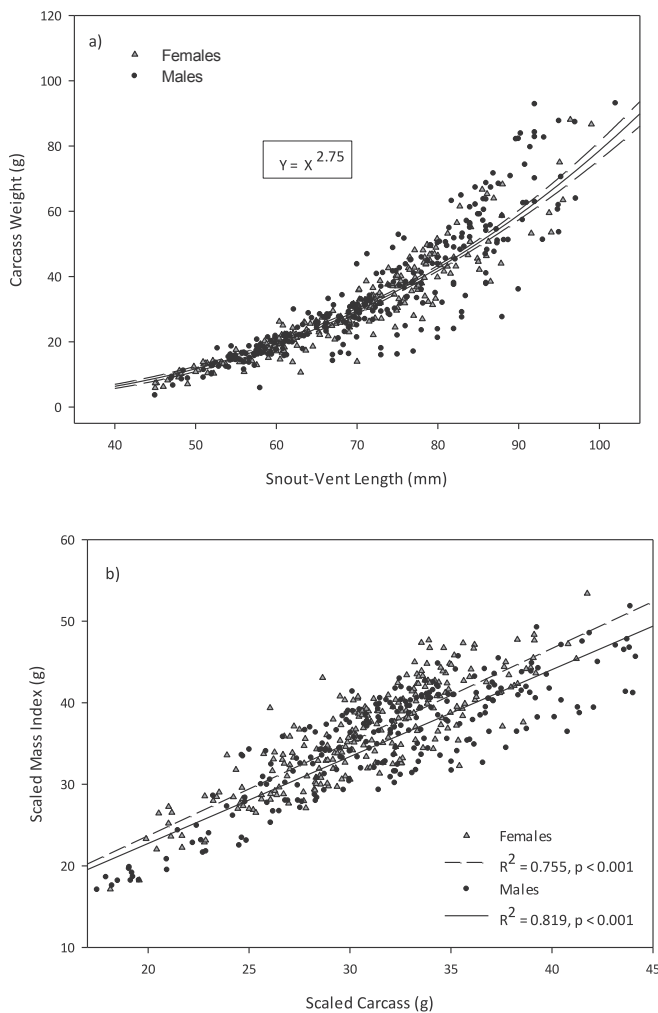
Fig. 5. a) Relationship between carcass weight and snout vent-length of *L. latrans*. The mathematical equation describing the regression line (full line) is indicated in the box. Dashed lines represent the 95% confidence interval of the regression line. b) Relationship between scaled carcass index and scaled mass index of a) females and b) males *L. latrans*. The linear regression curve obtained for each sex is illustrated. Slopes were significantly different amongst sexes ($p < 0.05$).

curve calculated between SCI and body condition was significantly different amongst sexes (Fig. 5b). Overall, SCI explained most of the variance inherent to the SMI: 75.5% in females and 81.9% in males (Fig. 5b).

3.5. Seasonal variation of the somatic indices

It is notable how SMI was considerably stable in females throughout spring and summer months, even though both SFI and SGI varied over 4–5 orders during this period. This is because the variation in SFI and SGI was perfectly opposite, SGI being at its maximum in the spring when SFI was near zero, and reaching it lower values in February-March when SFI increased (Fig. 6a). Overall, SMI decreased by about 14% over springtime (October–November), remained stable for most of the summer, and increased again by about 14% towards the end of summer (February - March). Variations in SCI closely followed the pattern above described for SMI (Fig. 6a). For its part, SLI exhibited a 30% drop from October to December, but this loss was completely recovered in the second half of summer (December–March) (Fig. 6a).

Seasonal variations observed in the somatic indices of the males presented many similarities with those observed in females, although

the amplitude of the changes was usually smaller (Fig. 6b). Similitudes with females included the fact that SGI and SFI varied in an opposite manner, SGI being elevated in the spring and reduced at the end of summer; whereas the opposite was true for SFI. However, the amplitude of those variations was in the order of 2–3 times the initial values, in contrast to the 4–5 times order of change observed in females. Interestingly, the variation is SLI was almost identical to that of females, SLI exhibiting a 30% reduction in the spring and early summer to later recover completely by the end of summer (Fig. 6b). Overall, the main difference between males and females resides in the fact that the SMI of males slowly decreases by 18.6% from October to December, whereas, in females, SMI decreases by 14% more rapidly, between October and November. Nevertheless, both sexes recover to initial SMI values by the end of the summer (Fig. 6).

4. Discussion

Amphibians are in decline around the globe and it is critical to improve and expand monitoring studies to obtain clear and precise information on the health and abundance of the different species. In this sense, the measurement of somatic and condition indices is a valuable addition to monitoring programs, as these indices inform on the general well-being of the individuals. Considering there is now increasing evidence showing that the scaled mass index (SMI) method of Peig and Green (2009; 2010) is the best approach for expressing frog body condition (MacCracken and Stebbings, 2012; Brodeur et al., 2020), the current study was designed to determine the correct manner of informing frog liver, fat, gonad and carcass somatic indices. Results obtained demonstrated that, in the case of *L. latrans*, the weight of all examined tissues and organs vary in an allometric manner in function of SVL, which implies that, to be adequate, calculated somatic indices should take into account the scaling of growth. In other words, this means that simple percentage ratio index, in which organ weight is divided by body weight and multiplied by 100, are inappropriate to express the somatic indices of *L. latrans* and the SMI method of Peig and Green (2009, 2010) should be employed.

In view of this conclusion, the scaling exponent of the power function describing the relationship existing between tissues and organ and SVL was defined, as this is an essential parameter when employing the SMI method. In the case of liver, fat and carcass, the non-linear regression curves were not statistically different amongst sexes and so a single relationship was described for both males and females *L. latrans*. Logically, the relationships between SVL and male and female gonad weight varied on distinct scale, and so it was necessary to analyze ovaries and testis separately. To allow a meaningful comparison between individuals of different sizes, a somatic index must be independent of body size and remove, through standardization, the effects of ontogenetic growth on the weight-length relationship (Peig and Green, 2010). This means that, to be of use, somatic indices should be uncorrelated with body size. (Labocha et al., 2014; Peig and Green, 2010). As we previously observed for *L. latrans* SMI (Brodeur et al., 2020), a weak but significant negative relationship was observed between all four examined somatic indices and SVL when the indices were calculated using the indirectly calculated bSMA proposed by Peig and Green (2009, 2010). However, if the somatic indices were calculated using an exponent *b* value calculated through a non-linear regression, the indices were independent from SVL. Again, as was proposed by Brodeur et al. (2020) for *L. latrans* SMI, these observations suggest that the indirectly calculated *b*SMA does not completely independisize SMI from the structural size of the animal, and is therefore not the best approximation of *b*. Overall, it appears to be more efficient to estimate the scaling exponent *b* directly through a non-linear regression than indirectly through calculating bSMA, as this allows to obtain a size-independent SMI value.

In view of all above exposed observations and results, the use of the SMI method is recommended for expressing L. *latrans* somatic indices,
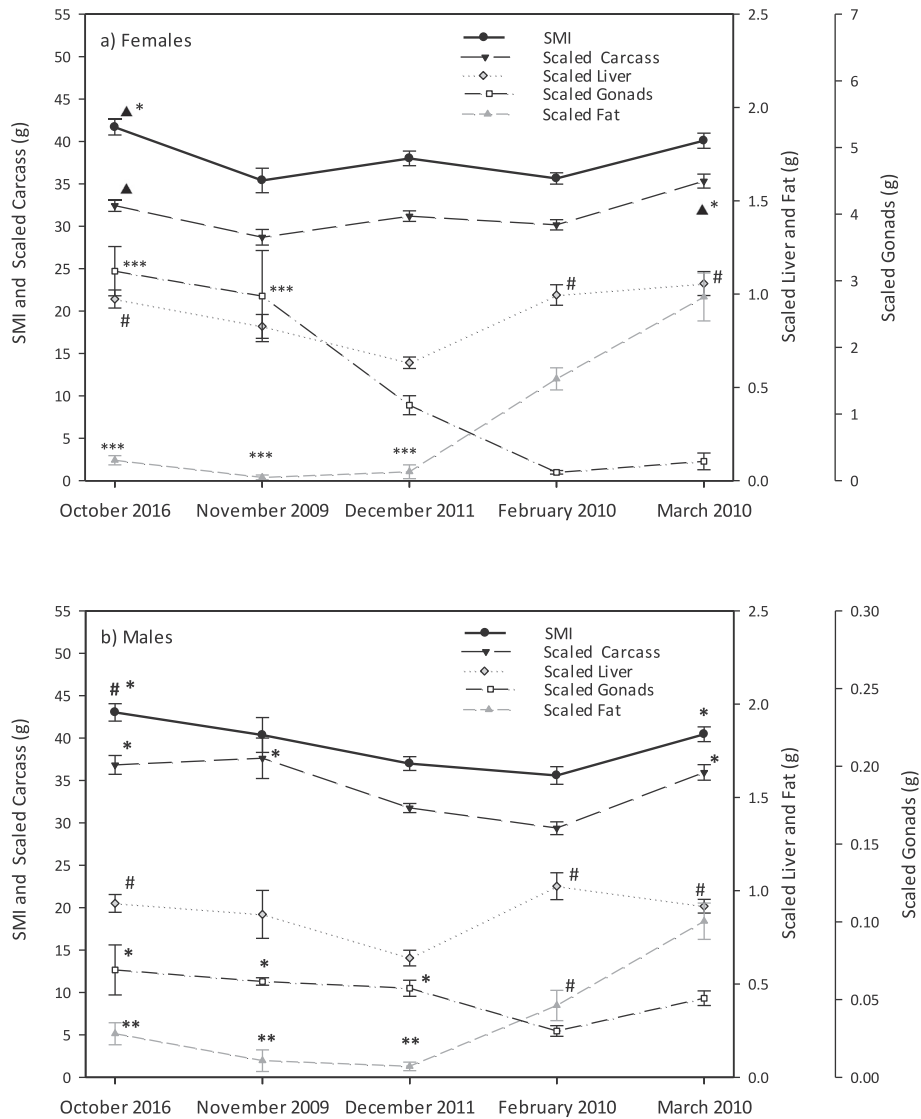
Fig. 6. Seasonal variation of the scaled mass index (SMI), and the scaled carcass, liver, fat and gonad indices of a) female and b) male *Leptodactylus latrans*. ▲ = significantly different from November, # = significantly different from December, * = significantly different from February, ** = significantly different from March, *** = significantly different from both February and March.

and scaling factors equal to 5.03, 3.11 and 2.75 are recommended to respectively estimate fat, liver and carcass scaled indices (SFI, SLI and SCI, respectively). In the case of the scaled gonadal index (SGI), the use of scaling factors equal to 3.81 and 6.49 are recommended to calculate male and female indices. Above mentioned scaling factors were employed in the current study to calculate the somatic indices of *L. latrans* through the SMI method in order to further examine the seasonal variation of the different indices as well as their relationship with body condition.

For both males and females, the variation in SFI and SGI was perfectly opposite: SGI was at its maximum in the spring, before breeding, when SFI was near zero because fat reserves had been used for basal metabolism and vitellogenesis during the winter. SGI then reached a low in mid-summer after the breeding season, which was apparently in part fueled by liver glycogen in both sexes, judging on the decrease in SLI observed from October to December. From this point on, food resources began to be principally converted to glycogen and lipid energy reserves so that SLI and SFI increased over the rest of the summer, while SGI remained low. This general pattern of seasonal energy variation is quite common in amphibians from temperate regions (Pider et al., 1992), and the inverse relation between abdominal fat bodies mass and

gonad mass has frequently been reported in female frogs (Rastogi et al., 1983; Prasadmurthy and Saidapur, 1987). Nevertheless, although a seasonal variation in testis weight is common in male frogs (Smith, 1950; Delgado et al., 1989; Huang et al., 1997), the pattern of inverse variation with body fat has been less frequently reported (Rastogi, 1976).

Interestingly, in spite of the fact that SGI and SFI varied over two to five orders during the spring-summer period, SMI was considerably stable and varied respectively by only 14 and 18% in females and males over the corresponding period. This is because the variations in SGI and SFI were perfectly opposite and compensated each other. In other words, body condition varied only slightly even though an important redistribution of energy reserves took place between the fat bodies and the gonads over the examined period.

This phenomenon also explains the fact that the overall changes in SFI and SGI correlated little with SMI, these somatic indices explaining only between 4 and 21% of the inherent variability of body condition. In contrast, body condition correlated much better with SLI, which explained about 50% of its variation. It is interesting to note that the better correlation observed between SLI and SMI does not mean that the liver was the organ presenting the greatest variations in energy

reserves, but rather that it was the organ which energy variations reflected best the variations in overall body condition.

A carcass index was calculated in the present study because cutaneous and subcutaneous fat bodies (carcass fat) and the skeletal muscle, which accumulates glycogen, may represent significant sites of energy accumulation in amphibians. Indeed, although fat bodies constitute an important storage site for lipids in anurans, measurement of the mass or lipid content of these organs alone may underestimate by as much as 50% the total amount of lipid available for metabolism (Wygoda et al., 1987). The present study confirms the importance of these carcass-related energy reserves for amphibian metabolism by showing that SCI vary very closely with body condition. Indeed, SCI correlated best with SMI and explained 75–80% of is variation. This observation also means that the traditional approach for validating a body condition index, which consists in examining its correlation with body fat contents may not be adequate for amphibian species (Schulte-Hostedde et al., 2005; Falk et al., 2017), as only a fraction of the energy reserves is considered when weighing the fat bodies.

In conclusion, the present study demonstrates the necessity to use scaled somatic indices to consider the allometric growth of the different tissues and organs in the frog *L. Latrans*. The study also highlights the fact that it is more efficient to define the scaling exponent through a non-linear regression of mass on length rather than by performing a standardized major axis regression of lnweight on lnlength. Moreover, results show that a variation of body condition of about 14–18% naturally exists over the spring-summer season, and that SLI is the somatic index that best correlates with this natural variation in body condition. Carcass-related energy reserves are also of importance for body condition, while SFI and SGI present wide and opposite variations over the spring-summer season. From an applied point of view, these results provide critical information for the design of biomonitoring studies with *L. latrans* or other temperate frog species from two different aspects: 1) It informs on the amplitude and the timing of the natural cycles that exist in body condition and somatic indices that need to be considered when designing monitoring studies, 2) It illustrates the fact that no single somatic index can solely illustrate body condition because of the intricate relationship existing between SGI and SFI and the importance of carcass-related energy reserves. In view of all the above, body condition therefore comes out as the ideal monitoring endpoint when intending to acquire information on frog energy status, especially considering that it does not involve the sacrifice of the animal. Indeed, for the sake of amphibian conservation, non-destructive monitoring designs based on the capture and release of the animal, should be prioritized over destructive samplings. In any case, should somatic indices be required for specific applications, the present results on *L. latrans* suggest they should be expressed in a scaled manner.

CRediT authorship contribution statement

Julie Céline Brodeur: Conceptualization, Investigation, Formal analysis, Writing - review & editing. **Josefina Vera Candioti:** Investigation. **Maria Jimena Damonte:** Investigation. **Maria Florencia Bahl:** Investigation. **Maria Belen Poliserpi:** Investigation. **Maria Florencia D'Andrea:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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