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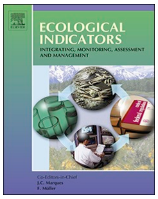

Frog body condition: Basic assumptions, comparison of methods and characterization of natural variability with field data from *Leptodactylus latrans*

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ABSTRACT

Body weight and snout-vent length (SVL) data of 3006 individual *Leptodactylus latrans* frogs collected over ten years in the Pampa Region of Argentina were analyzed to evaluate the best approach for expressing body condition and to characterize the natural variability of this parameter. Two different methods for expressing body condition were compared: the scaled mass index (SMI) and the residuals methods. Body weight of *L. latrans* was related to SVL through an allometric relationship described by the power function: $Y = 0.00006\ X^{3.11}$. The shape of the weight-length relationship was not affected by neither the sex of the animal nor its date or site of capture. A truly size-independent SMI value was more easily obtained when defining the scaling exponent through a non-linear regression of mass on length rather than when performing a standardized major axis regression of lnweight on lnlength. Overall, it was proved optimal to use a single scaling factor equal to 3.11 to compute SMI of all *L. Latrans* from the Pampa Region, irrespective of their gender and month or site of capture. Altogether, obtained results showed that SMI is a more performant indicator of body condition than residuals because it is less variable and it allows a better detection of effects. SMI and residuals condition factors deviated from each other in the extremities of *L. latrans* size range because scaling is not considered when calculating residuals. Body condition of females, males and juveniles was significantly lower in December/January compared to October/November and February/March. Moreover, juveniles consistently exhibited a lower body condition compared to both males and females. Based on the natural inter- and intra-annual variability observed for *L. latrans* SMI values, it should be possible to detect a 10% difference in SMI in an intra-annual study by sampling 14 to 17 frogs per group, whereas a 15% difference in SMI could be detected in an inter-annual study by sampling 23–29 animals per site. These numbers show that alterations of body condition should be straightforwardly identifiable in field studies with *L. latrans* when using SMI. The determination of SMI body condition factor during amphibian monitoring programs could be of great value, as it would provide information on amphibian health together with population abundance numbers.

1. Introduction

The body condition of an animal is an expression of its energetic status. An animal with a greater body condition is assumed to have more energy reserves than an animal with a poorer condition (Schulte-Hostedde et al., 2005; Labocha et al., 2014). Body condition is an important key variable in many ecological and ecotoxicological studies because it is closely related to an animal's health, vigor and fitness (Peig and Green, 2010; MacCracken and Stebbings, 2012). Indeed, the ability to accumulate energy reserves is a strong determinant of individual fitness, and individuals with larger energy reserves (and better body condition) are likely to have better fasting endurance, disease

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resistance, reproductive output and survival than individuals with smaller energy reserves and condition (Komoroski et al., 1998; McCauley et al., 2000; Schulte-Hostedde et al., 2005; MacCracken and Stebbings, 2012; Falk et al., 2017).

Body condition is difficult to measure directly, and any attempt at doing so necessarily involves the destruction of the whole specimen. For this reason, body condition is frequently estimated through an indirect non-destructive index based on the relationship between body mass and the total length or other morphometric measure of the animal (tarsus length, girth, etc.) (MacKracken and Stebbings, 2012). Such condition indices have been used in a variety of disciplines and taxa, ranging from insects to large mammals (Green, 2001). The ultimate goal of these condition indices is to use variations of body mass for a given body size as a surrogate for the variation in the size of the energy reserves and the individual's well-being (Peig and Green 2010). To be useful, a body condition index must accurately reflect energy stores and be independent of body size so that condition of different size animals can be adequately compared (MacKracken and Stebbings, 2012).

Over the last decades, much debate and controversy have surrounded the use and selection of body condition factors (Labocha et al., 2014). Discussions regarding body condition indices are normally concerned with the two essential attributes mentioned above which are: (1) if the condition index truly reflects energy stores and (2) if the condition index is independent of body size. Regarding this last question, older condition indices, which were based on simple ratios of body weight on body length, were demonstrated to be of limited use because they depend on body size (Jakob et al.,1996). As an alternative, residuals of an ordinary least squares regression of mass on a measure of length were used as condition factors but, although this method was allegedly validated by some (Schulte-Hostedde et al., 2005; Ardia, 2005), it was also amply criticized by others (Green, 2001; Garcia-Berthou, 2001; Freckleton, 2002). Over the years, it became clear that the problem of both ratio- and residuals-based condition factors is that they do not properly take into account the allometric scaling of growth: i.e. the fact that the relative proportion of different components and the relative length of different body parts change as total body size increases (Kotiaho, 1999; Marshall et al., 1999; Green, 2001). This is because 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). In order to be useful, a condition factor must thus control not only for body size, but for growth effects as a whole and their consequences on scaling (Peig and Green, 2009).

To resolve this issue, Peig and Green (2009) introduced a new 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 = $\text{aLength}^{\text{b}}$; with a and b as constants (Hoppeler and Weibel, 2005). In this equation, b is known as the scaling exponent and determines the dimensional balance between body mass and body length. If animal growth was isometric, the scaling exponent b would be equal to 3, as mass would correlate perfectly to the cube of the body length. However, because growth is allometric in most animals, the scaling exponent between body mass and body length usually varies between 2.5 and 3.2 in vertebrates (Green, 2001). The SMI method consists in quantifying the scaling exponent b for the studied species, 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. The scaling factor b (or bSMA) is species specific (or sex specific for dimorphic species), and body conditions based on SMI can be compared among groups sharing the same b value, regardless of variation in growth rate between individuals, populations or sex (Peig and Green, 2010).

An integrative measure of environmental quality, body condition is a valuable tool for conservation and environmental health assessment. Condition factors indeed serve to summarize the health and physiological state of animal populations as they depend on a wide variety of interacting biotic and abiotic environmental influences such as density, parasite load, predation risk, habitat loss, pollution, and food quality, quantity and availability (Stevenson and Woods, 2006; Cavraro et al., 2019). Traditionally employed as environmental health and quality indicators in fish (Teubner et al., 2014; Plourde et al., 2015; Cantafaro et al., 2017; Brodeur et al., 2017) and bird studies (Ackerman et al., 2019; Gladalski et al., 2019), condition factors are now used in a variety of taxa from invertebrates (Welden and Cowie, 2016) to top predators and marine mammals (Harwood et al., 2015; Dey et al., 2016). Among vertebrates, amphibians are the most threatened and rapidly declining group (Stuart et al., 2004; Pounds et al., 2006; Roelants et al., 2007). More than 40% percent of the amphibian species are believed to be threatened globally (Stuart et al., 2004; IPBES, 2019). Six major threats are believed to be involved in amphibian declines: habitat loss and fragmentation, commercial over-exploitation, introduced species, environmental contaminants, global climate change, and emerging infectious diseases, especially the chytrid fungus, *Batrachochytrium dendrobatidis* (Bishop et al., 2012).

In the context of a global amphibian crisis, it is necessary to develop, extend and improve monitoring programs of amphibian populations to provide critical up to date information on the status and health of amphibians worldwide. The inclusion of a body condition factor within the frame of a monitoring scheme would be of great value to such programs, as it would provide information on amphibian health in conjunction with population abundance numbers. The use of condition indices is much more recent and less expanded in amphibians than in other vertebrates. Until now, previous research on amphibians has relied almost exclusively on condition factors based on weight-length ratios (Bell et al., 2004; Waelti and Reyer, 2007; Park, 2008; Neveu, 2009; Attademo et al. 2011; Zhelev et al., 2017; Bionda et al., 2018; Otero et al., 2018) or residuals (Judge and Brooks, 2001; Leary et al., 2004; Hettyey et al., 2009; Băncilă et al., 2010; Brodeur et al., 2011; 2012; Hegde and Krishnamurthy, 2014; Guerra and Aráoz, 2016; Vera Candioti et al., 2019). A limited number of recent studies have used SMI to estimate body condition in anuran and urodeles amphibian species (MacCracken and Stebbings, 2012; Sanchez et al., 2014; Alvarado-Rybak et al., 2018; Romano et al., 2018).

MacCracken and Stebbings (2012) demonstrated that SMI accurately reflects amphibian energy stores in tadpoles and juveniles of both anuran and urodele species experimentally starved in a laboratory experiment. These results are promising and make a strong case for the need to further examine the potential uses and limitations of SMI as a bioindicator of amphibian energy reserves in conservation and environmental studies. Indeed, many aspects of SMI condition factors need to be evaluated in field conditions before SMI can be confidently and adequately employed as a bioindicator. For example, it is necessary to fully understand the influence of sex, age and season on the scaling of the length-weight relationship to determine whether the same scaling factor b can be applied in all cases. Furthermore, it is necessary to understand the importance of these factors on SMI and body condition itself to correctly interpret results obtained in the field. Finally, it is essential to have a clear understanding of the natural seasonal and inter-annual variation of SMI values for each amphibian species examined to correctly design, perform and interpret field monitoring studies.

In this context, the objectives of the current study were 1) to generate baseline information on the influence of sex, age, month and site of capture on the shape of the length-weight relationship of the South American common frog *Leptodactylus latrans*, 2) to determine which b scaling factor is the most adequate to use in each case when calculating SMI, 3) to examine whether SMI values are independent from body size, 4) to compare body condition data obtained with the SMI vs the residuals methods, 5) to evaluate the influence of sex and month of capture on the body condition of *L. latrans*, and 6) to determine the natural range of intra-annual and inter-annual variations of SMI values in *L. latrans* from the Pampa region of Argentina. This information is of importance at both regional and global levels. At the regional level, the information obtained will promote and improve the use of *L. latrans* as a bioindicator of the impacts of intensive agricultural production on the regional herpetofauna (Brodeur and Vera Candioti, 2017). At the global level, this study will provide critical information on the advantages, limitations and correct usage of SMI and residuals condition factors in anurans, as it is, to our knowledge, the first time these indicators are evaluated and compared in an anuran species in field conditions.

2. Methods

2.1. Studied species

Formerly known as *Leptodactylus ocellatus,* the South American common frog is now named *Leptodactylus latrans* (Lavilla et al., 2010). It is a large (120–140 mm) long-legged neotropical anuran widely distributed east of the Andes, from Venezuela to Argentina (Heyer et al., 2010). It occurs in a wide variety of habitats, including wet grasslands, riverbanks, tropical forests and highly modified agroecosystems (Heyer et al., 2010). Semi-aquatic*, L. latrans* is frequently found resting at the margin of water bodies and jumps into the water if disturbed. It is a generalist consumer whose diet consists of beetles, arachnids, lepidoptera, ants, annelids and other smaller anurans (Lajmanovich, 1996; Maneyro et al., 2004).

Mature males typically have large well developed arms and two conical spines on the first finger of the hand. Although *L. latrans* has been mentioned as one of the few amphibian species in which males are larger than females (Gallardo, 1987), a recent study did not find any sex differences in either size or growth pattern (López et al., 2017). 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). In temperate climates, reproductive activity may be observed throughout spring and summer (September to February), although it is occurs mostly in the spring (September) (López et al., 2011; Sánchez et al., 2013). In females, both plasma 17b-estradiol and gonadosomatic index show high values during the autumn-winter period, reaching a peak in august and decreasing the rest of spring-summer (Mosconi et al., 1996).

2.2. Frog morphometric data used

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 (Fig. 1). 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. Most of the data used for this study have never been published, but a small proportion was included in a previous publication (Brodeur et al., 2011). In all cases, frogs were captured by hand at sunset during spring and summer months of the southern hemisphere, which extend from October to March. 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 (Acculab Vic-303, precision = 0.001 g). Snout-vent length and body weight were measured on 3322 frogs. As recommended by Peig and Green (2009; 2010), 60 of these frogs were removed from the analysis because they clearly had a lower than average body weight for their length (maybe because they were sick or had parasites). Of the remaining frogs, only those with a SVL ranging between 44.7 a 105 mm were retained in the analysis to avoid that extremely small or extremely large frogs influenced too drastically the tails of the regression curves. Amongst the excluded frogs, 210 were very small young of the year individuals, which did not contribute to the definition of the scaling factor because their weight-length data were located on the flat left part of the curve. In total, 3006 *L. latrans* individuals were considered in the analysis.

2.3. Condition factors calculated

2.3.1. Residuals

The "residuals" method to express body condition was employed according to Schulte-Hostedde et al. (2005). In this method, an ordinary least squares regression of mass on snout-vent length is performed to define the regression line that establishes the theoretical body weight of an individual of a given length. Then, the theoretical body weight of sampled frogs (obtained by introducing the length of the animal in the equation of the regression line) is subtracted from the measured body weight to obtain the so-called "residual" value. Individuals with positive residuals are considered to present a good condition whereas individuals with negative residuals are regarded as having low energy reserves.

2.3.2. Scaled mass index

The SMI method to express body condition was computed according to Peig and Green (2009). The SMI method consists in first quantifying the scaling exponent b from the function $\text{Mass} = a\text{Length}^{b}$ for the studied species, and then calculating the predicted body mass of studied individuals at a given length. In the present study, b was estimated in two different ways to identify the most efficient approach: 1) it was directly modeled 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 scalled "bSMA" as defined by Peig and Green (2009)). SMI was calculated for the SVL corresponding to the average SVL of all sampled frogs.

2.4. Statistics

Non-linear power function regressions were realized on SVL and body weight data, and ordinary least square linear regressions were conducted on natural logarithmic transformations of SVL and weight data using the SigmaStat statistical software included in the SigmaPlot version 12.5 software (SPSS, Chicago, IL, USA). Generalized linear models (GLM) were used to evaluate the influence of sex and month of capture on the slope of the relationship existing between SVL and body weight. Natural logarithmic transformations of body length and weight data were used to ensure linearity. Monthly variations were examined by grouping animals captured in October and November (oct/nov), December and January (dec/jan), and February and March (feb/march) so as to represent, spring, early summer and late summer captures. The full model was first fitted:

$$\text{LNWEIGHT} = B_{o} + \text{Length} + \text{Sex} + \text{Month} + \text{Sex}*\text{LNLength}*\text{Sex} *\text{LNLength}*\text{Month} + \text{Month}*\text{Sex},$$

and the significance of the covariate, factors, and interactions were tested with an F-test. Next, non-significant interactions were removed from the model and significance of the remaining terms was examined using the F-test after fitting the new model. This sequential removal of non-significant terms was continued until only significant terms were left in the final model. GLM analysis was performed using Systat 11 software package and model validation was realized by controlling, through the Durbin-Watson D Statistic, that the dispersion of the residuals was normal.

SMI and residuals values were compared amongst size classes through a non-parametric Kruskal-Wallis one-way analysis of variance on ranks followed by Dunn's multiple comparison procedures because normality and equal variance of the data could not be obtained. For the same reason, the influence of sex and month on SMI and residuals was

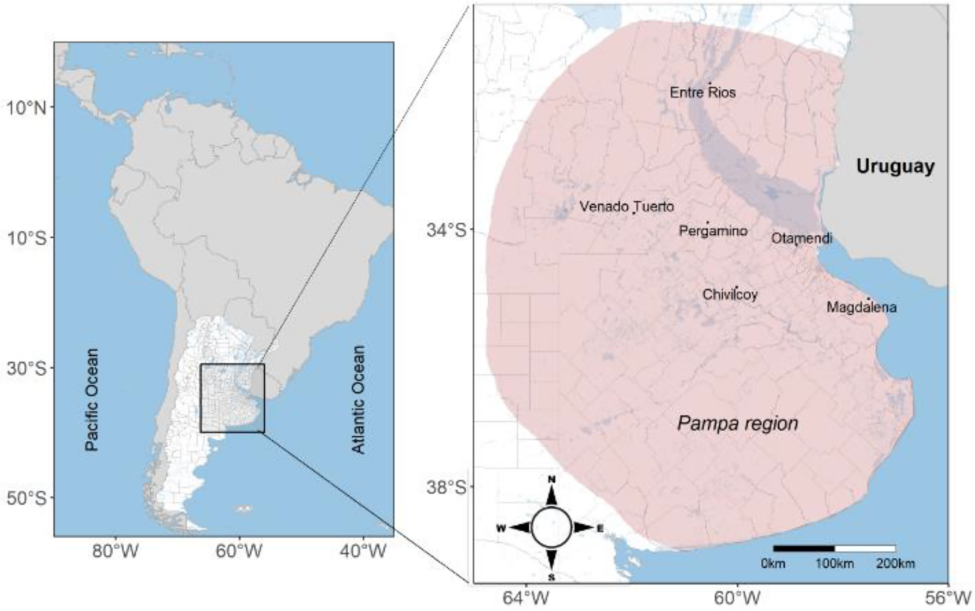

Fig. 1. Geographic locations within the Pampa Region of Argentina of the six locations where *L. latrans* frogs were sampled in the present study.

evaluated separately amongst sexes (males, females and juveniles) and months of capture (October/November, December/January, February/March) through the same approach: A Kruskal-Wallis one-way analysis of variance on ranks followed by Dunn's multiple comparison procedures. All regressions, analyses of variance and multiple comparison tests were conducted 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. 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.

Intra-annual variability in SMI was estimated by calculating, for frogs sampled in Magdalena, all monthly means included in a given spring-summer period, and then computing all possible differences amongst those means. Similarly, inter-annual variability in SMI was estimated by calculating, for frogs sampled in Magdalena, the monthly means of a given month for every spring-summer period for which this month was sampled, and then computing all possible differences amongst the means. The variability values calculated in this way were averaged over the periods considered in each analysis (intra-annual: spring-summer periods; inter-annual: months) and expressed as percent of the overall mean SMI. Using the largest standard deviations calculated for intra-annual and inter-annual variation of SMI in frogs from Magdalena, the sample size required to detect a 10 to 25% difference in SMI was calculated for statistical powers of 90 and 95% using the "*t*-test sample size" function of the SigmaStat statistical software included in the SigmaPlot version 12.5 software (SPSS, Chicago, IL, USA).

3. Results

3.1. Relationship between body weight and snout-vent length: Influence of sex, month and site of capture

Body weights of analyzed frogs ranged between 5 and 147 g, with an average of 30.43 ± 0.35 g (mean ± S.E.). SVL of captured frogs ranged between 27.51 and 145 mm, with an average of 66.34 ± 0.23 mm (mean ± S.E.). Males were slightly, but significantly, larger than females, the two groups respectively presenting an average SVL of 75.4 ± 0.16 and 71.7 ± 0.3 mm. The mean SVL of juveniles was 55.1 ± 0.16. The largest captured males measured 145 mm of SVL and weighed 157.6 g, while the largest female measured 128 mm and weighed 108.6 g. These larger animals were found in Entre Rios, at the northern limit of the study zone. When frogs from all sexes, locations and capture dates were considered, body weight and SVL of *L. latrans* presented an allometric relationship described by the following power function: $Y = 0.00006\ X^{3.11}$. Confidence intervals (95%) of the calculated scaling factor (3.11) ranged between 3.07 and 3.14. The natural logarithmic transformations of SVL and body weight values were linearly related through the equation $Y = 3.15\ X - 9.92$.

The scaling factors defining the power functions relating SVL to body weight were not statistically different amongst sexes (male, female or immature) or month of capture (oct/nov, dec/jan, or feb/march) (Fig. 2a). In the same sense, a GLM analysis demonstrated that neither the sex of the frogs nor the month of its capture significantly influenced the slope of the linear relationship existing between Ln SVL and Ln body weight (Fig. 2b). Only when frogs were divided according to their location of capture was a statistically significant difference observed amongst the scaling factors describing the power function between SVL and body weight. The six locations could be divided into two different groups: Magdalena, Pergamino and Venado Tuerto composed the first group with scaling factors between 2.9 and 3.1, whereas the other group was composed by the locations Chivilcoy, Otamendi and Entre Rios, which had slightly greater scaling factors that ranged between 3.3 and 3.5. As this result suggested that two different scaling factors may exist for *L. latrans* based on geographic location, further analysis were performed in which the scaling factors of the two groups of locations were calculated and compared. However, when analyzed in this manner, the scaling factors exhibited by the two groups of locations were not statistically different. This finding suggests that the site differences initially detected are probably linked to the fact that some locations were less extensively sampled than others, causing a bias in the scaling factor calculated for those locations. In view of the absence of a clear effect of sex, month or site of capture on the shape of the SVL-body weight relationship, it was concluded that a single scaling factor of 3.11 can be used for calculating SMI in *L. latrans* of the Pampa region.

3.2. Estimating body condition through scaled mass index or residuals method

As mentioned above, the scaling between SVL and body weight was

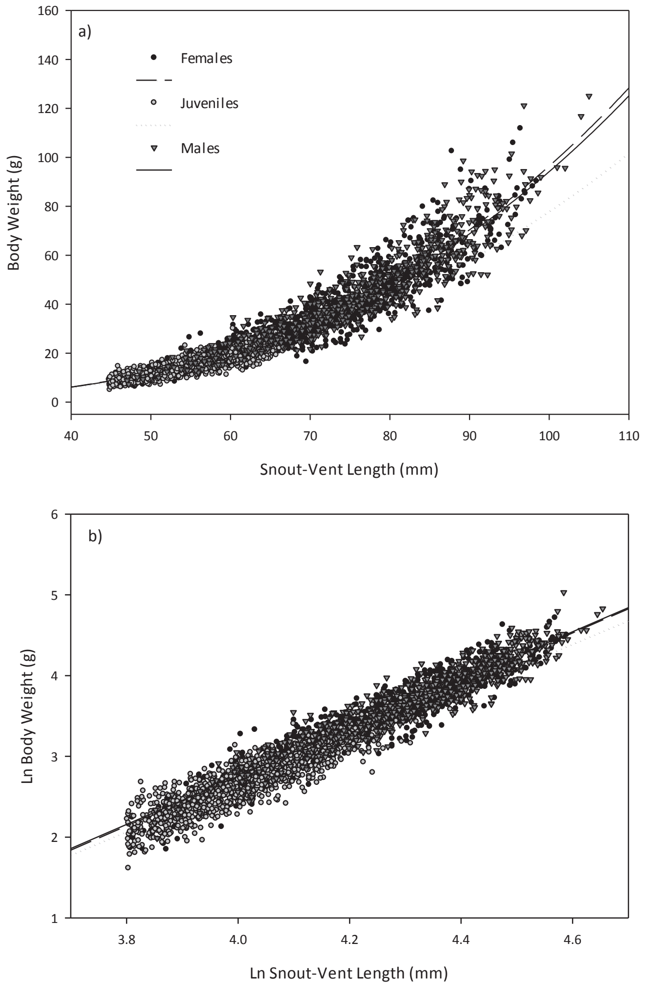

Fig. 2. Relationship between (a) body weight and snout-vent length and (b) natural logarithmic transformations of body weight and snout-vent length of *L. latrans* frogs from different sexes.

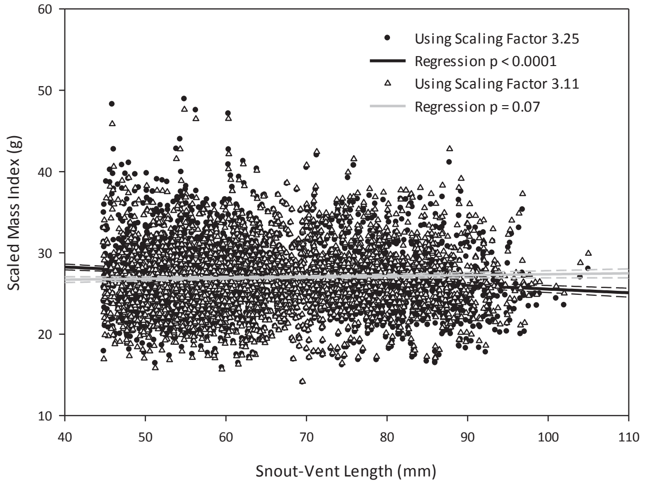

Fig. 3. Relationship between the scaled mass index and snout vent-length of *L. latrans* when the scaled mass index is calculated using a scaling factor of 3.25 or 3.11.

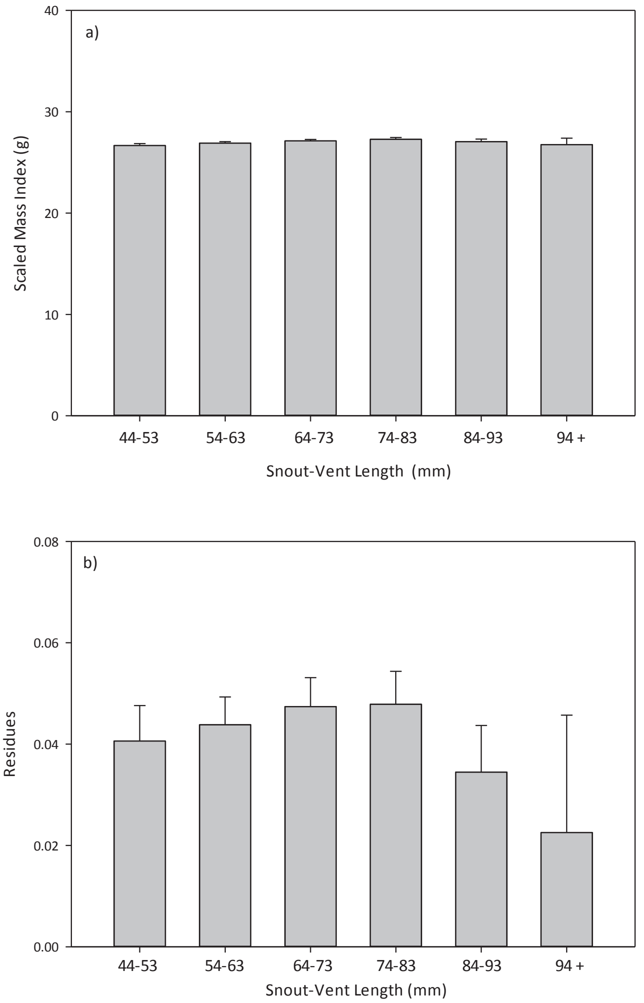

Fig. 4. Body condition (mean ± S.E.) of *L. latrans* of different size classes expressed as (a) scaled mass index (3.11 was used as the scaling exponent) and (b) residuals. No statistically significant difference was observed in either cases amongst the different SVL classes evaluated.

calculated as 3.11 when performing a non-linear regression of body weight data on SVL. In contrast, a scaling exponent of 3.25 was obtained if scaling was estimated as the standard major axis exponent (bSMA) and calculated indirectly by dividing the slope of an OLS regression of Ln-transformed weight and SVL data by the Pearson's correlation coefficient (as described in Peig and Green, 2009). When SMI was calculated using a scaling exponent of 3.25 (i.e. using the indirectly calculated bSMA), a weak ($m = -0.0452$; $R^2 = 0.017$) but significant ($p < 0.0001$) negative relationship existed between SMI and SVL; demonstrating that the scaling factor did not completely make SMI independent from the structural size (Fig. 3). In contrast, if SMI was calculated using a scaling exponent of 3.11 (i.e. using the estimation from a non-linear power function regression), the SMI was independent from SVL and the regression between these two parameters was not significant ($p = 0.0788$) (Fig. 3). Considering that a body condition index must be independent of age classes and body size to be of utility (Peig and Green, 2009), a scaling factor of 3.11 was considered optimal for SMI estimation in *L. latrans* and was used in further analysis.

Body condition estimated through the residuals method was also independent from SVL ($p = 0.664$; Pearson correlation). The independence of body condition from body size when expressed both as SMI (calculated with 3.11 as a scaling factor) and residuals is illustrated in Fig. 4 where no significant difference is observed in either cases amongst the different SVL classes evaluated. Nevertheless, Fig. 4 also highlights the fact that SMI is a much more stable and less variable parameter than the residuals; the standard error of the means being proportionally much smaller in the case of the SMI. As regards the relationship observed between the two estimates of body condition, Fig. 5

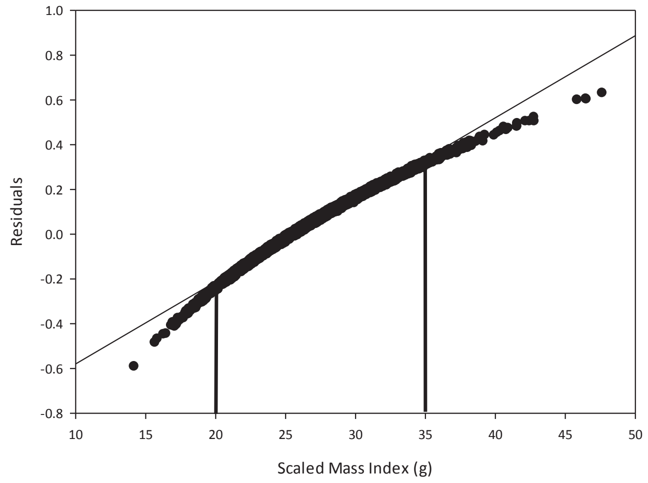

Fig. 5. Relationship between *L. latrans* body condition values estimated with the residuals or scaled mass index methods (3.11 was used as the scaling exponent). The interval between the bold lines corresponds to the range of values whithin which the two condition factors vary linearly.

shows that SMI and the residuals co-vary proportionally for frogs between 20 and 36 g (approximately 60 and 72 mm based on the length-weight relationship). Outside of this range, the variation in residuals associated to a 1 g change in SMI is respectively greater or lower than the change observed within the 20–35 g range: it is greater in animals smaller than 20 g and lower in frogs larger than 35 g (Fig. 5). The lack of coincidence of the two body condition measures in the extremity of the size range is due to the fact that scaling is not taken into account when calculating residuals.

Finally, Fig. 6 illustrates how sex and month of capture influence body condition when calculated as SMI or residuals. With both condition measures, the condition of females, males and juveniles was significantly lower in dec/jan compared to oct/nov and feb/march; all individuals showing equivalent body condition during these last two periods. Moreover, when condition was defined in terms of SMI, juveniles consistently exhibited a lower condition than both males and females, which, for their part, did not differ significantly (Fig. 6a). The lower condition of juveniles was, however, less clearly detected when using residuals, as a difference was only detected with males, and this only in dic/nov and feb/march; the difference with females going undetected (Fig. 6b). This distinct capacity of the SMI and residue methods to detect variation in smaller size juveniles is likely linked to the nonlinear relationship present between the two measures in animals smaller than 60 mm (Fig. 5). This observation shows that the two methods are not equivalent and that SMI is more performant because it takes into account the allometric nature of *L. latrans* growth.

3.3. Intra-annual and inter-annual variability of body condition in L. Latrans

The natural intra-annual and inter-annual variabilities in the body condition of *L. latrans* were characterized by examining the SMI of animals captured in Magdalena on the different months of various spring-summer seasons extending over 10 years, from 2006 until 2016 (Fig. 7). Although statistically significant differences were observed amongst the different months of every spring-summer seasons considered; it was difficult to detect a distinct pattern of monthly variations, as the direction of the inter-month variations differed amongst seasons (Fig. 7). Inter-annual variation of the SMI values measured on a given month was on average 12.3%, which was greater than the observed average intra-annual variability of 7.6% (Fig. 7; Table 1). The importance of the inter-annual variability is exemplified by SMI values measured in 2013–2014, which are greater than that observed in

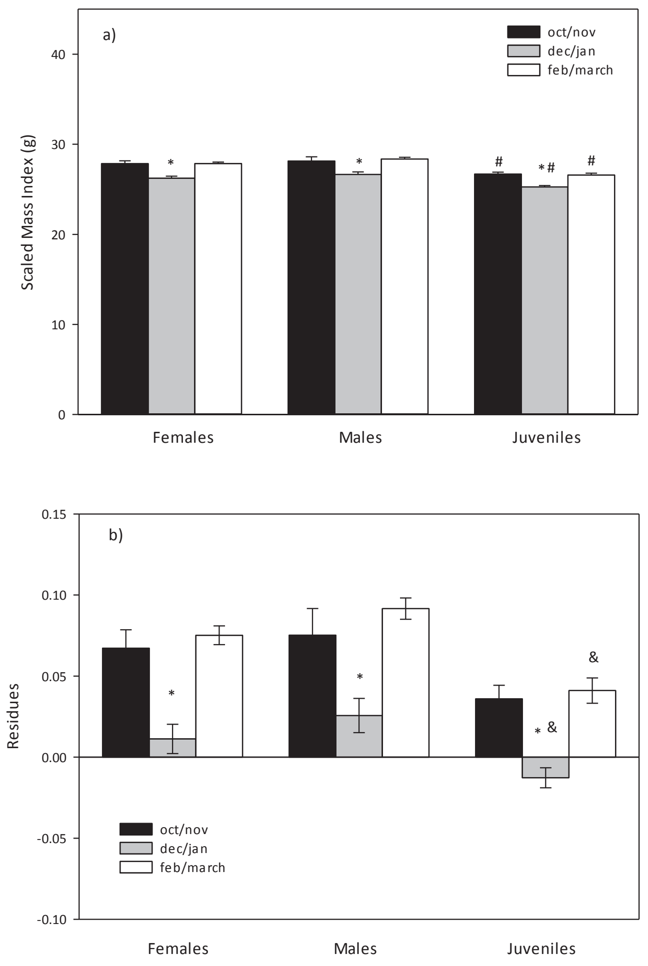

Fig. 6. Body condition (mean ± S.E.) of *L. latrans* of different sexes and capture date expressed as a) scaled mass index (3.11 was used as the scaling exponent) and b) residuals. * = different from same sex frogs captured on other months, # different from males and females captured on the same months, & = different from males captured on the same months.

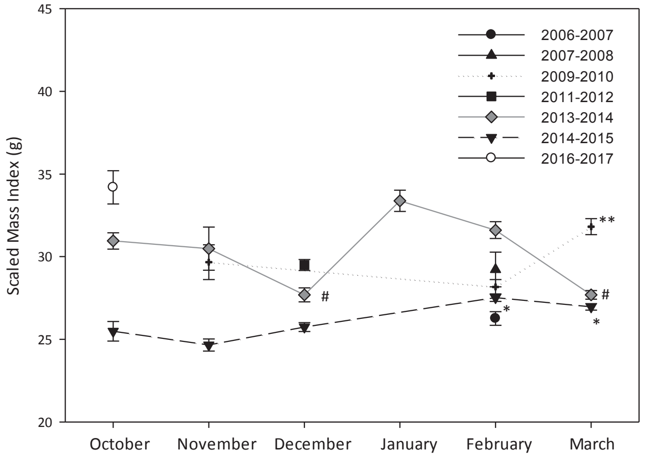

Fig. 7. Scaled mass index of *L. latrans* sampled on the different months of various spring-summer seasons in the natural reserve “El Destino”, Magdalena, Buenos Aires, Argentina. In the southern hemisphere, spring-summer seasons extend from October of a given year until March of the following year. ** = greater than February in 2009–2010, # = lower than October, January, February (and November in the case of March) in 2013–2014, * = greater than November and December (and October in the case of February) in 2014–2015.

Table 1
Inter-annual and intra-annual variation of the scaled mass index calculated for *L. latrans* captured in Magdalena on the different months of various spring-summer seasons extending over 10 years, from 2006 until 2016.

Inter-annual	**October**	**November**	**December**	**February**	**March**
Mean SMI (g)	30.2	28.3	27.5	28.2	28.8
Standard Deviation of Mean SMI (g)	4.41	3.15	1.74	2.5	2.6
Variability (%)	19.2	13.8	8.4	8.7	11.2
Intra-annual	**2009–2010**	**2013–2014**	**2014–2015**		
Mean SMI (g)	29.8	30.3	26.1		
Standard Deviation of Mean SMI (g)	1.84	2.25	1.16		
Variability (%)	8.15	9.0	5.5		

Table 2
Sample sizes required to detect a 10 to 25% difference in SMI considering observed inter-annual and intra-annual variation. Type I error considered (α) = 0.05.

Inter-annual				
Difference in SMI	*10%*	*15%*	*20%*	*25%*
90% Power	51	23	14	9
95% Power	62	29	17	11
Intra-annual				
Difference in SMI	*10%*	*15%*	*20%*	*25%*
90% Power	14	7	5	4
95% Power	17	9	6	4

2014–2015 in almost every month considered (Fig. 7). Inter-annual variability was greater in October and November, with 19.2 and 13.8%, respectively, whereas the spring-summer season of 2013–2014 presented the largest intra-annual variability in SMI, with 9% (Table 1). Using the largest standard deviations calculated for intra-annual and inter-annual variation in SMI of frogs from Magdalena, the sample size required to detect a 10 to 25% difference in SMI was calculated for statistical powers of 90 and 95% (Table 2). Sample sizes from 17 to 4 individuals were estimated sufficient to detect a difference in SMI between two groups, in an intra-annual context (within a given spring-summer season) (Table 2). Comparatively, sample sizes from 62 to 9 individuals are required to detect a difference in SMI between two groups, in an inter-annual context (between two different spring-summer seasons) (Table 2).

4. Discussion

In the present study, males of *L. latrans* were on average 4 cm larger than females, as previously reported by Gallardo (1987). Nevertheless, in spite of this size difference, the sex of the frog did not influence the shape of the relationship between SVL and body weight (Fig. 2). This similitude in the length-weight relationship means that, although sexual size dimorphism is present, the sexes have a similar body design, and therefore a similar scaling factor *b*. The current study furthermore demonstrates that, within the Pampa Region of Argentina, neither the date nor the site of capture significantly modifies the weight-length relationship of *L. latrans*. Altogether, these findings indicate that a single scaling factor may be used to characterize the exponential mass gain resulting from a size increase; and that SMI can be compared and computed in a similar manner in all *L. latrans* ranging between 27.5 and 145 mm, irrespective of their gender and month or site of capture. This conclusion only applies to *L. latrans* included within the above mentioned size range, and further studies are needed to define whether it also applies to metamorphs and young of the year froglets.

To allow a meaningful comparison between individuals of different sizes, a condition factor 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, mean condition values should be equivalent for different age classes and be uncorrelated with body size. (Labocha et al., 2014; Peig and Green, 2010). In the present study, when SMI was calculated using the indirectly calculated bSMA of 3.25, a weak but significant negative relationship was observed between SMI and SVL (Fig. 3). However, if SMI was calculated using a *b* value of 3.11, calculated through a non-linear regression, the SMI was independent from SVL (Fig. 3). Similar to what is reported here with frogs, Falk et al. (2017) also observed a weak, but significant, correlation between SMI and length in the Burmese python when indirectly calculating *b* as *b*SMA. 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 the above-described findings, it is recommended that a scaling factor *b* equal to 3.11 be employed when calculating SMI in *L. latrans* from the Pampa Region. SMI values produced in this way are both uncorrelated with SVL and equivalent amongst all size categories of frogs (Fig. 3 and Fig. 4a). Although body condition values expressed as residuals also satisfy these requirements (Fig. 3 and Fig. 4b), Fig. 4 shows that residuals are a much more variable parameter than SMI. The importance and utility for field assessments of the lower variability of body condition values when expressed as SMI vs residuals is exemplified in Fig. 6, where SMI allows detecting differences between juvenile frogs and males and females adult frogs, that otherwise go undetected with residuals. Indeed, juvenile animals were found to exhibit a significantly lower body condition than both males and females at all sampling dates when condition was expressed as SMI, but, when condition was expressed as residuals, a difference was only detected with males and only at two out of three sampling dates. Aside from the greater variability of the residual values, the fact that the two condition indices do not vary proportionally at the extremes of the size range of *L. latrans* (Fig. 5) was also likely at cause in the lower capacity of residuals at detecting differences in juvenile's body condition. This is because residuals, to the contrary of SMI, do not take into account the allometric nature of frog body growth (Peig and Green, 2009; 2010). Altogether, obtained results show that SMI is a more performant indicator of body condition than residuals in the frog *L. latrans*. This conclusion is similar to what has now been observed by a number of authors in a variety of other animal groups (Peig and Green, 2009; 2010; Labocha et al., 2014; Falk et al., 2017)

It is interesting to note that a significantly lower body condition was detected in juvenile frogs (Fig. 6) even though SMI was equivalent amongst all size classes of frogs (Fig. 4). This indicates that the low body condition of juvenile frogs is truly related to the state of sexual maturity of the frog and is not a scaling effect. Alternatively, this observation also illustrates the importance of using a condition factor that is completely independent of body size to compare animals of different sizes. Juveniles can be defined as sexually immature post-tadpole animals. In the present study, juveniles presented an average SVL of 55.1 ± 5.4 (mean ± S.D.), indicating that they were included in the two or three lower size classes considered in Fig. 4. This observation is similar to values reported by Maneyro et al. (2004) which found that juvenile *L. latrans* had a SVL between 50 and 75 mm. Most probably, the low body condition of juvenile frogs is linked to the fact that these animals have not yet began to accumulate energy reserves and gonad tissue in prevision for breeding. However, very little information is available in the literature on juvenile frog energy budget, body condition and energy reserves to further explain or describe this phenomenon.

When all animals were considered, a statistically significant decrease in body condition was observed in dec/jan compare to oct/nov and feb/march; body condition being equivalent during these two periods (Fig. 6). This pattern of variation is consistent with the general pattern of changes in energy reserves that is often described in amphibians living in seasonal environments: Energy reserves gradually decline over winter months, are rapidly depleted during spring breeding, after which they reach a low, but they increase again towards the end of summer and early autumn (Pider et al., 1992). This clear pattern of seasonal variation was, however, less obvious when frogs were examined at a single site (Magdalena) and sampling year (Fig. 7). This fact exemplifies the natural intra-annual and inter-annual variability that may exist in body condition. As stated above, amphibian energy reserves and body condition are strongly linked to their reproductive cycle, which is itself largely dependent on climate. It is therefore easy to understand that naturally occurring year to year variations in monthly climate trends will generate some level of variability in the timing of seasonal variations in body condition. Furthermore, although *L. latrans* breeds mainly in the spring, the species has also been reported to breed throughout spring and summer (López et al. 2011; Sánchez et al., 2013), which will also add variability to the seasonal variations in body condition.

Looking at 10 years of SMI data from Magdalena, the site that was most frequently sampled in the current study, average inter-annual variability in SMI was estimated at 12.3%, which is more than the average intra-annual variability of 7.6%. Such information about the natural range of variability of body condition is critical for formulating field monitoring and sampling studies, but has rarely been provided for an amphibian species. When considering the scale of the natural variability in SMI body condition, it is possible to understand that intra-annual sampling designs will be more performant at detecting differences in body condition than inter-annual designs. Indeed, from data obtained, it is clear that the smallest detectable differences in body condition will be of about 10 and 15–20% for intra-annual and inter-annual designs, respectively. This reality can be visualized in Table 2, where the estimated sample size required to detect a significant difference in SMI is reduced at these levels of difference. The current study therefore shows that it should be possible to detect a 10% difference in SMI in an intra-annual study design by sampling 14 to 17 animals per group, whereas a 15% difference in SMI could be detected in an inter-annual design by sampling 23–29 animals per site. Overall, these numbers indicate that field measurement and monitoring of body condition is feasible and that good detection levels are possible to achieve in *L. latrans* when using SMI as a condition factor. The inclusion of a SMI body condition factor to amphibian monitoring programs could be of great value, as it would help provide information on amphibian health in conjunction with population abundance numbers. To facilitate and make more effective the use of SMI as a biomonitoring tool, further studies should be realized in other amphibian species so as to unveil species-specific information regarding the scaling factors and the natural variability of body condition values.

CRediT authorship contribution statement

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