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Original article

Reproductive effort and the egg number vs. size trade-off in *Physalaemus* frogs (Anura: Leiuperidae)

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ABSTRACT

Patterns of reproductive allocation are expected to differ between species according to temporally and spatially variable costs of reproduction. Even when reproductive allocation patterns are the same, species can also differ in how the reproductive effort is allocated between offspring number and size. In this study, we compared the reproductive allocation patterns and the offspring number vs. size trade-off in two frog species, *Physalaemus biligonigerus* and *P. gracilis*, using bivariate (standardized major axis) and multiple linear regressions. Both species showed a common slope between body size and reproductive effort and thus a similar allocation pattern although *P. biligonigerus* has a larger body size (shift along common slope) and makes a lower reproductive effort (shift in intercept) than *P. gracilis*. We suggest that similar allocation patterns may be related to the shared phenologies of these frogs and that the differences in reproductive effort could represent either an adaptive shift (e.g., change in body space for the clutch) or a historical constraint. There was a negative correlation between fecundity and egg size in *P. biligonigerus* but not in *P. gracilis* as predicted by the acquisition–allocation model (Y-model). This study constitutes the first valid test of the Y-model based on recent predictions derived for the trade-off between offspring size vs. number. We conclude that future studies should compare reproductive allocation patterns between species using tests of allometric slopes with appropriate phylogenetic control to detect both adaptive shifts in allocation strategies and correlations with other life-history traits.

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1. Introduction

The age-schedules of reproduction are optimal strategies of allocation for maximizing reproductive output in response to variable costs of reproduction during adult lifetime (Stearns, 1992; Roff, 2002). Three classical costs of

reproduction derived from the trade-offs between resources allocated to reproduction vs. growth, survival, and future reproduction, have been discussed in the literature: cost in terms of growth, cost in terms of survival, and cost in terms of future reproduction (Stearns, 1992; Nilsson and Svensson, 1996; Roff, 2002). The cost in terms of growth is especially

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important in organisms with indeterminate growth such as ectothermic vertebrates, which continue to grow after reaching sexual maturity, because larger body sizes are associated with higher reproductive output (Reiss, 1989; Heino and Kaitala, 1999). Moreover, costs in terms of growth and survival have been shown to be additive or interactive in a model of allocation to reproduction in two fish species and the differences in allocation pattern were best explained by considering differential costs in terms of survival between species (Roff et al., 2006). Therefore, the optimal pattern of allocation to reproduction in a species is a result of the combined costs of reproduction during lifetime, and species with different costs related to their specific natural history will exhibit differences in allocation patterns (Reiss, 1989; Waelti and Reyer, 2007). These differences in allocation patterns can be assessed with an allometric analysis of phenotypic variables which, in spite of limited explanatory power (Stearns, 1992; Roff, 2002), have proven useful in suggesting the mechanisms underlying reproductive allocation tactics in animals (Reiss, 1989; Loison and Strand, 2005; Herreras et al., 2007) and in plants (Shibuya et al., 2007; Vilela et al., 2008).

When comparing allometric relationships between reproductive effort and body size ('reproductive allometry'), a difference in slopes implies a change in allocation patterns between populations or species. For example, stronger costs of reproduction early after maturation would favour a pattern of allocation with a steeper slope and a lower y-intercept (Fig. 1A), and on the other hand, lower costs would favour higher reproductive efforts in young females (i.e., flatter slope and higher y-intercept, Fig. 1A). A shift along the common slope indicates

that both species share the same allocation pattern and relative reproductive effort (RRE) (Fig. 1B) and differences can be accounted for by body size alone (Loison and Strand, 2005). When y-intercepts are different (shift in the "reproductive" axis), the species/populations differ in their RRE independently of body size (Fig. 1C). Finally, shifts along the common slope and in the y-intercept suggest that the difference in RRE is at least partially explained by body size (Fig. 1D).

After a female has spent a defined amount of resources in their offspring, a trade-off results from how those resources are split between the number and the size of offspring. The offspring number vs. size trade-off has been a classical topic in animal life-history theory at intra- and interspecific levels (Stearns, 1992; Roff, 2002; Kolm et al., 2006). Whereas a trade-off should theoretically occur always as long as resources are limited (Bernardo, 1996), many species do not demonstrate a negative relationship between egg number vs. size in natural or laboratory conditions (Uller and Olsson, 2005; Hořák et al., 2008). In fact, van Noordwijk and de Jong's (1986) model, also called the Y-model (de Jong and van Noordwijk, 1992), predicts that even when a trade-off occurs between two traits, a positive correlation between them can arise when there is variation in acquisition of resources among individuals. Christians (2000) adapted the model for the egg size vs. number trade-off and interpreted that a negative correlation will be observed only when the variation in acquisition of resources is lower than the variation in allocation of that investment. Although several empirical studies have supported this modified model (Christians, 2000; Brown, 2003; Uller and Olsson, 2005; Herreras et al., 2007), Roff and

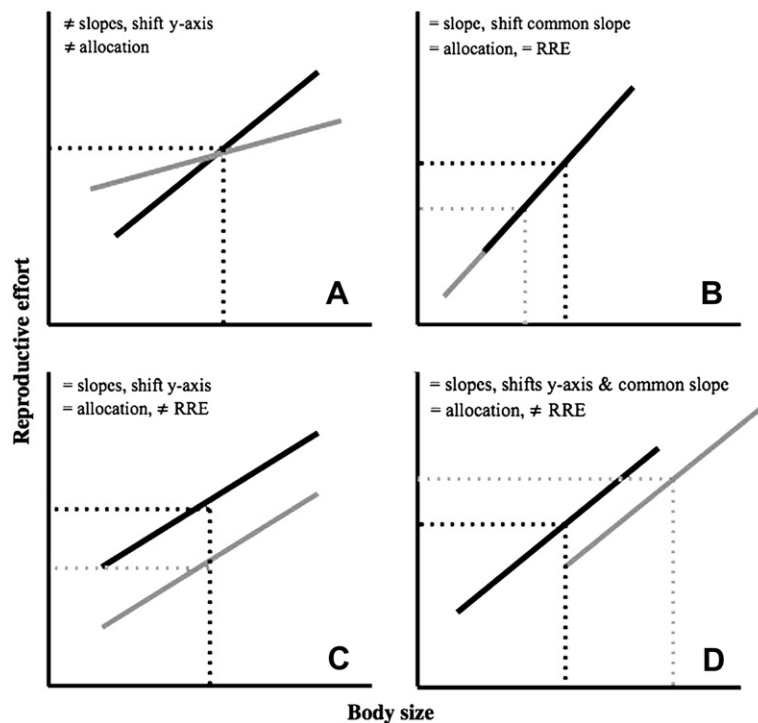


Fig. 1 – Comparison of reproductive allocation patterns between species/populations based on differences in allometric slopes and in shifts along the reproductive axis and the common slope. (A) Different slopes and shift in intercept, (B) common slope but shift in intercept (reproductive axis), (C) common slope but shift along common slope, and (D) common slope with both shifts in intercept and along the common slope. Abbreviation: RRE = relative reproductive effort (see text).

Fairbairn (2007) have recently argued that the original model was misinterpreted and led to invalid empirical tests of its predictions. Roff and Fairbairn (2007) clarified that, according to the Y-model, the sign of the correlation between offspring number vs. size depends not only on the variances of resource acquisition and allocation but also on their mean values. As far as we know, only one study has tested the Y-model with the predictions derived by Roff and Fairbairn (2007) for the trade-off between reproductive vs. somatic allocation (Crnokrak and Roff, 2002), but no test has been performed for the offspring number vs. size trade-off.

Anurans are a convenient model for studying reproductive allocation because in most species, the female's reproductive effort is spent in the egg clutch and there is no parental care, which simplifies quantification of reproductive parameters. Most importantly, some previous studies have shown high variation in clutch partitioning and in reproductive effort between and within species (Crump and Kaplan, 1979; Berven, 1988; Lips, 2001; Lüddecke, 2002). Other studies have found high mortality rates during reproductive events implying a significant cost of reproduction (Lemckert and Shine, 1993). Amphibians are typical indeterminate growers that have to confront a cost in terms of growth when resources are invested into reproduction (Halliday and Verrell, 1988; Jørgensen, 1992). In addition, anurans are capital breeders where high reproductive output depletes energy reserves and affects future reproductive expenditures (Nilsson and Svensson, 1996; Doughty and Shine, 1997; Bonnet et al., 1998). All of these factors should make amphibian life histories ideal for studying changes in patterns of allocation as a response to temporal and spatially variable costs of reproduction.

Amphibians, and particularly anurans, have received little attention in life-history studies compared to other ectothermic vertebrates such as fishes and reptiles (Kozłowski and Teriokhin, 1999 and references therein; Shine, 2005) and a limited number of studies have assessed single-species patterns of reproductive allocation in laboratory or field conditions (Ryser, 1989; Tejedo, 1992; Lemckert and Shine, 1993; Miaud et al., 1999; Buschmann, 2002; Lüddecke, 2002; Lyapkov et al., 2002; Lardner and Loman, 2003; Castellano et al., 2004; Lauck, 2005). Whereas most studies found a significant positive relationship between reproductive effort with body size, usually only r^2 and p -values are reported but the regression equation parameters (slope and intersect) are rarely compared. Instead, studies usually report mean estimates of reproductive effort in different population or species, which renders comparisons of allocation patterns between species because individual variation is ignored (Kuramoto, 1978; Tarkhnishvili, 1993; Kusano and Hayashi, 2002; Severtsova et al., 2002; Prado and Haddad, 2005). Thus, there has been a generalized lack of integration between intra- and interspecific analyses of reproductive allocation even though the simultaneous analysis using allometric scaling may help to elucidate the proximal causes of reproductive allocation patterns (Loison and Strand, 2005).

In this study, we analyze the variation in reproductive allocation in female *Physalaemus biligonigerus* (Cope, 1861) and *P. gracilis* (Boulenger, 1883) using an allometric analysis of body size and reproductive effort. Comparing the allometric slopes of reproductive allocation between these species and other

traits of their reproductive biology, we aim to suggest the possible key adaptive shifts associated with their allocation patterns. We also test the Y-model for the trade-off between egg number and egg size based on the predictions derived from Roff and Fairbairn (2007).

2. Materials and methods

2.1. Data collection

Adult females of *P. biligonigerus* were collected monthly with pitfall traps in Campos de COFUSA, Departamento de Rivera (30° 53' S, 55° 33' W) between March 2001 and February 2002. Females of *P. gracilis* were collected in Espinas creek, Departamento de Maldonado (34° 47' S 55° 22' W) between September 1998 and April 2000 (Camargo et al., 2005). Both species are abundant and their distributions are almost allopatric in Uruguay (Langone, 1995; Núñez et al., 2004). Although they display similar reproductive modes and advertisement calls (Kwet, 2002), they belong to different species groups: *P. biligonigerus* to the *P. albifrons* group and *P. gracilis* to the *P. gracilis* group (Nascimento et al., 2005). Both species reproduce in ephemeral and semi-permanent shallow ponds usually with abundant submerged vegetation and produce a spherical foam nest that floats on top of the water. Except for reports about advertisement calls, reproductive periods, and characteristics of the egg clutch (Ceï, 1980; Langone, 1995; Kwet and Di-Bernardo, 1999; Zaracho et al., 2005; Bionda et al., 2006; Achaval and Olmos, 2007), little is known about the reproductive ecology of these species (but see Camargo et al., 2005). Both species are listed in the non-threatened, least concern category by IUCN Red List criteria (IUCN et al., 2006).

Individuals were fixed with formalin and ovaries were extracted to examine the maturational status of oocytes. Only mature females with fully developed oocytes were used in this study by visually confirming the presence of equal-sized, yellow-pigmented oocytes. Females with white-colored, translucent oocytes, or with signals of atresic oocytes (visible as dark spots on ovaries) were considered immature individuals and discarded from this study. Out of 59 *P. gracilis* females collected in Maldonado, 37 were considered as matured females and from 145 *P. biligonigerus* females caught in Rivera, 22 were selected for subsequent analysis. Ovaries were extracted and weighed to estimate reproductive effort (RE) based on the wet ovarian mass and the ova counted to quantify fecundity (F). Mean ovum mass was estimated as the reproductive effort divided by the fecundity ($OS = RE/F$). Subsequently, female carcasses were weighed to estimate wet body mass and snout-vent length was measured to quantify body length. All individuals are stored in the Vertebrate Zoology Collection of Facultad de Ciencias, Universidad de la República, Uruguay (ZVCB).

2.2. Data analyses

2.2.1. Allocation to reproduction

Variables were log-transformed and input into SMATR ver. 2.0 software (Falster et al., 2006) to estimate standardized major

axis (SMA) regression slopes. Linear regression is appropriate for estimating the correlation, testing the association, and predicting the y variable from the x variable, but the SMA method is preferred for estimating the line-of-best-fit (slope), which is the parameter of interest in allometric analysis (Warton and Weber, 2002). When the x variable is measured with error, the slope of a linear regression will be a biased estimate of the relationship between x and y because residuals are minimized in the y -axis only (Warton et al., 2006). We estimated the SMA relationship between body mass (BM) and reproductive effort (RE) to assess allocation patterns in each species: $\log \text{BM} = \log a + b(\log \text{RE})$. We also estimated SMA slopes for the relationship between fecundity vs. body mass and ovum mass vs. body mass. In order to compare body shape between species, we estimated the SMA relationship between body mass and snout–vent length and used ANCOVA to test for differences in body mass using snout–vent length as a covariate.

We tested for significant allometry assuming the null hypothesis that $b = 1$ and performed three types of slope comparisons between species in SMATR. First, SMATR estimated a common slope and performed a test of heterogeneity of slopes between species (Fig. 1A). When a common slope cannot be rejected, SMATR tests for shifts along this common SMA slope (Fig. 1B) and in elevation of slope (Fig. 1C) between species via ANOVA comparisons of transformed variables (Falster et al., 2006). The number of iterations was set to 10,000 and the critical p -value to 0.05. We calculated the SMA residuals ($y - bx$) and tested the assumption of normally distributed residuals (Warton, 2006) with a Shapiro–Wilks test in Statistica 5.0 (Statsoft, 1999).

2.2.2. Trade-off between fecundity and offspring size

The trade-off between number and size of ova was evaluated with a multiple linear regression between fecundity as the dependent variable and mean ovum mass and body mass as the independent variables. Multiple regression evaluates the correlation between the variables of interest (fecundity and ovum size in this case) while controlling for the covariate (body mass). Finally, we calculated variances in $\log \text{OS}$, F , and RE for each species to assess the fit to the Y-model using the equation derived by Roff and Fairbairn (2007): $\text{covariance OS and } F = \frac{1}{2} [\text{variance } \log \text{RE} - (\text{variance } \log \text{OS} + \text{variance } \log F)]$. The model predicts that when the variance in investment (RE) is larger than the summed variances of the allocation components (OS and F), a trade-off between OS and F will not be detected because their covariance is positive.

3. Results

3.1. Reproductive phenology

The monthly trapping of mature *P. biligonigerus* females showed that reproductive activities started in October, peaked in November, and declined in December (Fig. 2). Similarly, the period of reproductive activity of *P. gracilis* occurred between October and December with a maximum in November (Fig. 2). Indeed, *P. gracilis* showed the same reproductive periods during 1999 and 2000 (see Camargo et al., 2005). The single-peak pattern of reproduction in both species suggests

that, as in most temperate frogs, the females were able to lay a single clutch per reproductive season.

3.2. Allocation to reproduction

Basic statistics for all the size and reproductive variables are shown in Table 1. The SMA relationship between snout–vent length and body mass was significant in *P. gracilis* [$b = 3.24$, 95%-confidence interval (CI) = 2.56–4.11] but not in *P. biligonigerus* ($b = 3.57$, 95%-CI = 2.30–5.54; Table 2). Since the latter regression was not significant, a test of slopes is meaningless and instead we used ANCOVA to test for body mass differences between species with snout–vent length as a covariate. Variables were log-transformed prior to analysis to meet assumptions of normality and variance homogeneity. There is no difference in snout–vent length ($t_{57} = -0.09$, $p = 0.93$) but *P. biligonigerus* is significantly heavier than *P. gracilis* ($F_{1,56} = 119.66$, $p < 0.001$; Table 1).

The SMA analysis found a significant relationship between reproductive effort and body mass in *Physalaemus gracilis* ($b = 3.24$, 95%-CI = 2.56–4.11) and marginally significant in *P. biligonigerus* ($b = 3.57$, 95%-CI = 2.30–5.54; Table 2, Fig. 3A). In both species, this relationship showed a positive allometry because their slopes were significantly different from $b = 1.00$ (Table 2). The test of common slope between species indicated that species do not differ in the scaling exponent b between body mass and reproductive effort (common $b = 1.91$, test statistic = 0.61, $p = 0.43$). The ANOVA test detected a significant shift in intercept between species ($F_{1,57} = 69.5$, $p < 0.01$) with *P. biligonigerus* having a larger mean reproductive effort than *P. gracilis* (Table 1). In addition, the ANOVA test also detected a significant shift along the common slope ($F_{1,57} = 38.5$, $p < 0.01$) with *P. biligonigerus* having a larger mean body mass than *P. gracilis* (Table 1). The residuals of the regressions were normally distributed in both *P. gracilis* (Shapiro–Wilks test: $W = 0.95$, $p = 0.11$) and *P. biligonigerus* ($W = 0.97$, $p = 0.68$). Only *P. gracilis* showed a significant and positive SMA slope between fecundity vs. body mass ($b = 1.19$, 95%-CI = 0.94–1.51) and ovum size vs. body mass ($b = 1.24$, 95%-CI = 0.92–1.67; Table 2). In both cases, the SMA slope was not significantly different from 1, which implies an isometric relationship between fecundity and body mass and between ovum mass and body mass (Table 2).

3.3. Trade-off between fecundity and offspring size

The multiple regression between fecundity, ovum size, and body mass was significant in both *P. gracilis* (whole model $r^2 = 0.48$, $F_{2,34} = 17.36$, $p < 0.001$) and *P. biligonigerus* (whole model $r^2 = 0.27$, $F_{2,19} = 4.80$, $p < 0.05$). When body mass is held constant, the relationship between fecundity and ovum size was not significant in *P. gracilis* ($\beta = 0.02$, $t_{24} = 0.12$, $p = 0.90$) but in *P. biligonigerus* was significant and negative suggesting a trade-off between egg number and size ($\beta = -0.56$, $t_{19} = -2.94$, $p < 0.01$). Plots of regression residuals vs. predicted values showed that multiple regression assumptions of linearity, normality, and homoscedasticity were met. The covariance between fecundity and ovum size, based on the variances of these variables and reproductive effort (Table 1), was positive for *P. gracilis* (covariance F , $\text{OS} = 0.008$) but

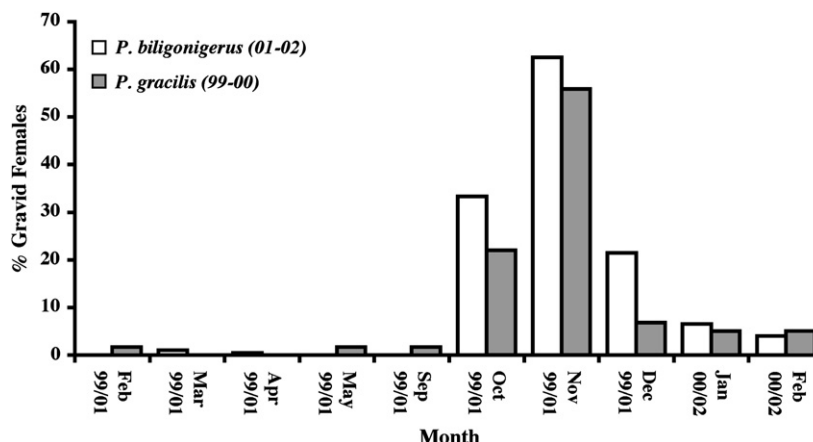


Fig. 2 – Monthly relative abundance of mature females captured in pitfall traps between March 1999 and February 2000 in Departamento de Maldonado (*Physalaemus gracilis*) and between March 2001 and February 2002 in Departamento de Rivera (*P. biligonigerus*). Females of *P. gracilis* collected between September 1998 and February 1999 were also included in SMA analyses.

negative for *P. biligonigerus* (covariance F , $OS = -0.015$). Standard deviation estimates of covariances based on bootstrapped resampling was ~ 0.009 for both species.

4. Discussion

Our primary focus was on detecting differences in reproductive allocation between species based on shifts in allometric slopes and in discussing possible factors, such as phenology and body shape, that may underlie those allocation patterns. However, we acknowledge that because we used a single population from each species, and given the geographic and temporal variation of amphibian life histories, it is likely that different patterns could occur in other populations (Miaud et al., 1999; Morrison and Hero, 2003). For example, a population of *P. biligonigerus* in Argentina produced between ~ 1000 and 1600 eggs (Zaracho et al., 2005) but in our population in Uruguay, fecundity varied between ~ 200 and 400 (Table 1). Similarly, *P. gracilis* in Brazil lays between 400 and 800 eggs (Kwet and Di-Bernardo, 1999) but in our study ranged between 700 and 1300 (Table 1). Second, sampling size could also limit statistical power to detect differences in allometric slopes because of the wide confidence intervals in our SMA relationships (Table 2). Finally, because we could not evaluate the complete energy budget with the data at hand, we could not directly test for trade-offs between growth and

reproduction but instead we inferred costs based on reproductive allocation patterns.

4.1. Reproductive phenology

Whereas previous reports have indicated that *P. gracilis* and *P. biligonigerus* reproduce from spring to summer (September/November–February/March; Langone, 1995; Achaval and Olmos, 2007), we found that their reproductive periods are shorter and restricted to the spring (October–December). A likely explanation for this discrepancy is that past reports have relied on male calling patterns only but we used the phenology of gravid females as a more accurate indicator of breeding activities. It is known that males of prolonged breeders start calling long before reproduction to increase chorus size (Camargo et al., 2005) but it is unclear why males may keep calling after most gravid females are gone.

4.2. Allocation to reproduction

Organisms with indeterminate growth show positive allometry in reproductive allocation because larger adults spend proportionally more surplus energy on reproduction than smaller individuals (Reiss, 1989). The significant positive allometry found in this study indicates that as females of both species become larger, they invest proportionately more in their offspring. Similar reproductive allocation strategies are

Table 1 – Basic statistics (mean \pm standard deviation) for body size and reproductive variables of *Physalaemus gracilis* and *P. biligonigerus*

Species	n	SVL (cm)	BM (gr)	RE (gr)	F	OS ($gr \times 10^{-4}$)
<i>P. gracilis</i>	37	2.99 ± 0.27	2.25 ± 0.66	0.27 ± 0.15	311.11 ± 103.91	8.49 ± 2.64
<i>P. biligonigerus</i>	22	3.00 ± 0.22	4.41 ± 1.16	0.34 ± 0.14	1020.59 ± 353.62	3.56 ± 1.52

Abbreviations: n = sample size, SVL = snout–vent length, BM = body mass, RE = reproductive effort (ovarian mass), F = fecundity, OS = mean ovum size (RE/F).

Table 2 – Standardized major axis (SMA) regression results and test of isometry for *Physalaemus gracilis* and *P. biligonigerus*

Variables species	SMA regression		Test of isometry		
	<i>a</i>	<i>r</i> ²	<i>p</i>	<i>F</i>	<i>p</i>
SVL vs. BM					
<i>P. gracilis</i>	-1.21	0.51	<0.01	153.97	<0.01
<i>P. biligonigerus</i>	-1.07	0.05	0.32	-	-
BM vs. RE					
<i>P. gracilis</i>	-1.29	0.52	<0.01	41.03	<0.01
<i>P. biligonigerus</i>	-1.55	0.15	0.08	6.51	<0.05
BM vs. F					
<i>P. gracilis</i>	2.07	0.51	<0.01	2.13	0.15
<i>P. biligonigerus</i>	2.24	0.04	0.39	-	-
BM vs. OS					
<i>P. gracilis</i>	-3.51	0.23	<0.01	2.19	0.15
<i>P. biligonigerus</i>	-4.68	0.05	0.34	-	-

Variables used in analyses were: snout-vent length (SVL), body mass (BM), reproductive effort (RE), fecundity (F), ovum size (OS). Abbreviations: *a* = intercept. Significant regressions are in bold.

indicative of similar costs of reproduction or alternatively, different costs are compensating each other to produce a similar net cost in both species. From the three basic types of costs studied in the literature: costs paid in growth, survival, and future reproduction (Stearns, 1992; Roff, 2002), we have no data for inferring which have a role in the patterns of allocation to reproduction except for indirect evidence supporting a cost paid in growth. Because larger females are more fecund than smaller ones in both species, then body size seems to be

constraining the space for the clutch and selecting against spending energy on reproduction at small body sizes. Indeed, this body size–fecundity advantage for large females has been argued as the selection pressure maintaining the usual female-biased sexual size dimorphism in frogs (Halliday and Tejedo, 1995).

We think that one possible reason for a common allometric slope in reproductive effort between species could be related to their activity patterns or phenology. Two opposite phenological patterns have been identified in frogs: prolonged breeders with an extended breeding period lasting several months and explosive breeders that display intermittent reproductive bouts limited to a few days in a breeding season (Wells, 1977). The long reproductive period of prolonged breeders makes them more vulnerable to predators, and in contrast, predators find it more difficult to prey upon explosive choruses composed of many synchronized calling males in a short breeding period (Stebbins and Cohen, 1995). Because predation risk seems to be an important cost associated with breeding in frogs (Lemckert and Shine, 1993), higher vulnerability of prolonged breeders to predators should increase their costs of reproduction in terms of survival at small body sizes. They will tend to display a pattern of reproductive allocation with a steeper slope (and lower intercept), whereas “explosive” breeders under lower costs in terms of survival would display patterns with a flatter slope (Fig. 1A). In contrast, species with similar reproductive phenologies would also show similar patterns of allocation to reproduction (similar slope) and this prediction could be supported by our study because both frog species displayed similar reproductive periods (Fig. 2) and similar allocation patterns (Fig. 3). A possible link between phenology and allocation patterns should not be surprising since reproductive season length is a key determinant

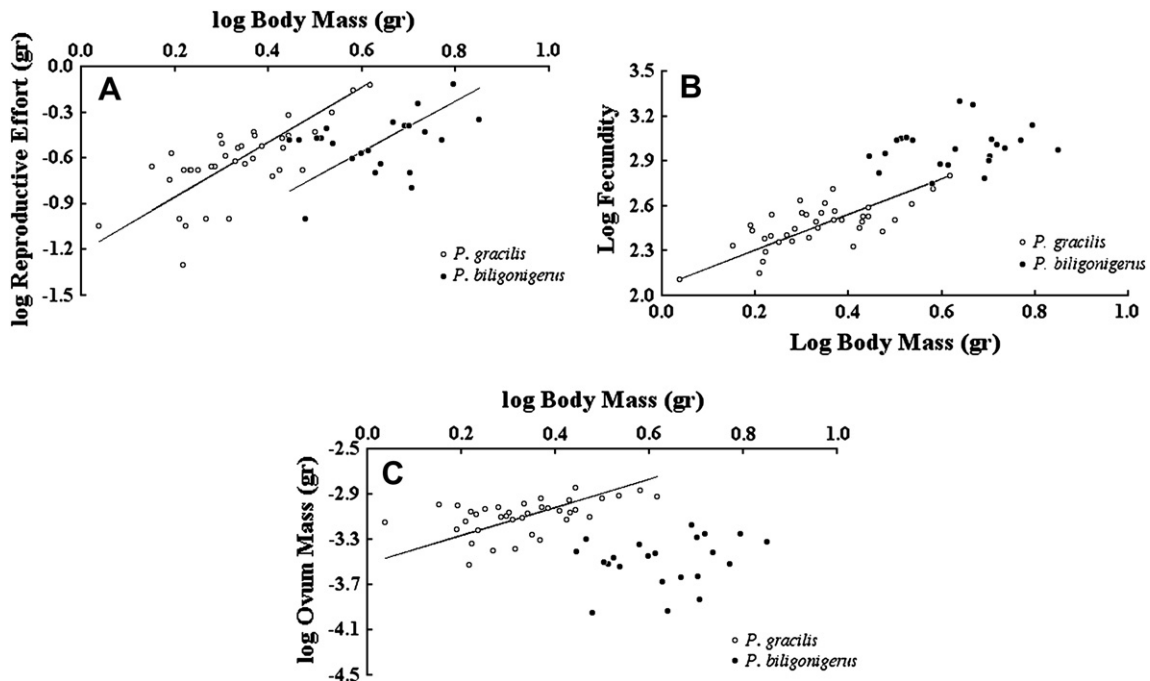


Fig. 3 – Standardized major axis regression in *P. biligonigerus* (filled circles) and *P. gracilis* (open circles) between (A) reproductive effort and body mass, (B) fecundity and body mass, and (C) ovum mass and body mass.

of other life-history traits in indeterminate growers living in seasonal environments (Kozłowski and Teriokhin, 1999).

A shift along the common slope reflects a scaling response in reproductive effort to differences in body size between populations (Fig. 1B, Loison and Strand, 2005). A shift in the intercept (shift in elevation or the reproductive axis), suggests a qualitative change in RE at all body sizes as a result of an adaptive transition and/or a response to a limiting constraint (Fig. 1C). In this study, the SMA analysis found shifts both along the common slope and in intercept (Fig. 1D), indicating that although *P. biligonigerus* has a larger mean RE than *P. gracilis* (Table 1), the RE of *P. biligonigerus* was lower than expected according to its body size. If there was no shift in intercept, *P. biligonigerus* would have a mean RE ~ 1 gr, based on a mean BM = 4.41 gr (Table 1) and the SMA slope of *P. gracilis*, but the actual mean RE is 0.34. Both allometric shifts suggest that the relatively low reproductive effort of *P. biligonigerus* compared to that of *P. gracilis* is not simply a consequence of different body sizes but an adaptive shift between species in other life-history traits (e.g., body shape, offspring survival, etc.) and/or a result of historical constraints. In fact, the stouter body aspect of *P. biligonigerus* in comparison with that of *P. gracilis* as indicated in the ANCOVA analysis supports the idea that an increase in somatic mass may be limiting the space available for the clutch. An investigation of other limiting factors will demand a comparative phylogenetic study of several *Physalaemus* species to distinguish between either independent or correlated evolution of clutch and body size in this genus.

4.3. Trade-off between fecundity and offspring size

Although both species showed a similar allocation pattern of reproductive effort, they showed contrasting patterns of clutch partitioning: *P. gracilis* invested in more and larger ova with increasing body size, but *P. biligonigerus* females produced either many small eggs or a few large eggs. The patterns are consistent with the predictions of the Y-model for the offspring number vs. size trade-off and suggest that species differed in levels of resource acquisition. As Roff and Fairbairn (2007) predicted, because the covariance between fecundity and ovum size in *P. gracilis* was positive, there was no evidence of a trade-off between these variables. In contrast, a trade-off was evident in *P. biligonigerus* because the covariance between offspring number and size was negative. As far as we know, this is the first study supporting the predictions of the Y-model as adapted for the offspring number vs. size trade-off by Roff and Fairbairn (2007). All past studies have invalidly tested the Y-model with the predictions derived by Christians (2000), which were shown to be oversimplified (Roff and Fairbairn, 2007). Our study found that there was high variation in reproductive effort among *P. gracilis* females that can be accounted for by differences in female quality such that some females invested proportionally in more and larger ova (Uller and Olsson, 2005). In fact, this is actually observed in our data because larger females produce more of bigger eggs as shown in the SMA regressions between BM and F (Fig. 3B) and between BM and OS (Fig. 3C). In contrast, the summed variances in egg size and egg number in *P. biligonigerus* exceeded the variation in clutch investment leading to the

observed trade-off which may have been caused by body size and/or resource limitations (Uller and Olsson, 2005).

5. Conclusions

We encourage that future studies compare the allocation patterns of reproductive effort and clutch partitioning in prolonged vs. explosive species using SMA tests of allometric slopes. Most studies to date have either compared mean estimates or reported allometric relationships separately for each species but without testing for shifts in allometric slopes (see Severtsova et al., 2002; Rodrigues et al., 2004; Prado and Haddad, 2005). New analyses of allocation patterns and clutch partitioning will have to include additional *Physalaemus* species to sample the diversity of reproductive strategies in the genus (Rodrigues et al., 2004; Brasileiro and Martins, 2006 and references therein; Maneyro et al., in press). In addition, a strongly supported hypothesis of phylogenetic relationships within the genus will be needed to take into account historical constraint on reproductive variables, to address evolutionary correlation with other life-history traits, and to identify key adaptive shifts in reproductive tactics.

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