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Interspecific patterns for egg and clutch sizes of African Bufonidae (Amphibia: Anura)

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ABSTRACT

Little is known about reproductive trade-offs in African amphibians, but such data, particularly in the form of quantitative measurements, are a key for investigating life history evolution. Here we compile and analyze known data on African bufonids from published material and new data from preserved museum specimens, to investigate interspecific patterns of egg and clutch sizes variation. Our data is a composite of mixed sources, including ova data from dissected females and laid clutches from observations in the field. Our study shows that, as body size increases, clutch size increases but egg size decreases, and when correcting for body size, egg size is inversely correlated with clutch size. These parameter interactions however, are different for different reproductive modes. In free-swimming larval developing species, the same trends are recovered, but for lecithotrophic viviparous species no significant correlations could be recovered for clutch size and body size nor for the trade-off between clutch size and egg size, and egg size is positively related to body size. The egg size of *Nimbaphrynoides occidentalis* (Angel, 1943) is a clear outlier, which may be due to its matrotrophic viviparous reproduction. In addition, we observed no statistical difference between ova data collected from dissections and laid clutch data from field observations, which suggests that such a mixed dataset has utility in comparative analyses.

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1. Introduction

Life history theory predicts that key ontogenetic processes such as the timing and allocation of reproductive efforts are strongly subjected to natural selection in favour of maximizing an individual's inclusive fitness. The study of life history theory is therefore largely concerned with understanding why such an immense variation in reproductive strategies exists in nature and whether an optimization hypothesis can always be recovered as the underlying explanation (Stearns, 2000). The trade-off between the number of offspring and parental investment per offspring for example has been the focus of many fundamental concepts in ecology and evolution (e.g. Lack, 1947; MacArthur and Wilson, 1967; Van Noordwijk and de Jong, 1986) and the size and number of eggs per clutch is known to vary strongly both within (Cummins, 1986; Williamson and Bull, 1995; Christians, 2002; Berven, 2008) and between (Kuramoto, 1978; Blackburn, 1991; Figuerola and Green, 2005; Martin et al., 2006) species.

For amphibians, relationships between egg diameter and the number of eggs per clutch are central measures used to characterize reproductive modes, along with oviposition site, rate and duration of development, size of hatchling and type of parental care (Salthe and Duellman, 1973). Already in 1886, Boulenger noted that terrestrially breeding amphibians generally have larger eggs, but lay fewer than their aquatic breeding counterparts (Boulenger, 1886). Since then, numerous other studies have investigated the interspecific relationship of egg and clutch size (e.g. Wake, 1978; Barbault, 1984; Hödl, 1990; Pupin et al., 2010); reviewed in (Duellman and Trueb, 1994; Wells, 2007), but African taxa tend to be underrepresented in broad scale comparative analyses (e.g. Summers et al., 2006; Wells, 2007; Gomez-Mestre et al., 2012), or are only the subject of studies that focus on a single taxon (Barbault, 1984; *Phrynobatrachus* Rödel and Ernst, 2002; *Boulengerula* Malonza and Measey, 2005). Here, we investigate interspecific patterns in clutch and egg size in relation to body size of true toads of Africa (Family Bufonidae) to test whether a trade-off exists between the two. Bufonids are interesting for this kind of study given the starkly contrasting breeding strategies they exhibit (e.g. Van Bocxlaer et al., 2010) and African bufonids specifically cover a particularly broad range of life history strategies, from large bodied, temporary pond breeders such as *Amietophrynus gutturalis* (Power, 1927) depositing

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tens of thousands of eggs (Channing and Howell, 2006) to the small, habitat specialist, matrotrophic viviparous toad *Nimbaphrynoides occidentalis* with extremely reduced clutches of no more than 17 eggs (Angel and Lamotte, 1944). Wells (2007) has shown that egg and clutch size relationships vary between different reproductive modes, but restricted his sampling to New World amphibian lineages. To test whether the same is true for African bufonids, we compare trends in life history parameters in species with free-swimming larva and species that give birth to live young in the form of lecithotrophic viviparity (formerly referred to as ovoviviparity; Blackburn, 1999) and matrotrophic viviparity.

Researchers working on Afro-tropical systems are often faced with problems of data availability and compatibility. Even when data are available, observations stem from varying types of quantitative and qualitative measures and composites of this nature are often needed to compile a suitable number of data points for meaningful analyses. We therefore also investigated whether egg counts and measurements taken from dissections of preserved, gravid females are comparable to data collected from field observations of laid clutches by testing whether trends observed for "ovarian clutches" are significantly different than those for "laid clutches".

2. Methods

2.1. Data collection

An exhaustive literature search for data on egg diameter and the number of eggs per clutch for African bufonid species was carried out. Information was compiled from primary literature indexed and searchable via Google Scholar (Google Inc., CA, USA) and webof-knowledge.com (Thomson Reuters, Zurich, Switzerland) and from library searches for un-indexed journals, books and field guides in personal literature collections and the library of the University of Basel as well as the Natural History Museum (NHM), London. In cases where the literature source did not explicitly state or otherwise infer how counts or measurements were obtained, data was assumed to refer to laid clutches, not dissections. Information on reproductive modes was obtained from the IUCN Red List online database (www.iucnredlist.org).

The literature dataset was complemented with new data collected for this study. The collections of the NHM and the Museum für Naturkunde, Berlin were visited and gravid females with visibly distended abdomens were dissected to retrieve the ovarian egg mass. Investigators were careful not to cause excessive damage to specimen, by either using pre-existing incisions (likely made by collectors to allow for preservatives to enter the body cavity) or by making incisions on only one side of the specimen, by cutting a crescent shape from just below the armpit along the flank towards the inguinal region. Eggs were gently lifted out of the body cavity with forceps and placed onto a glass plate and kept moist with 70% methylated spirit.

Information on the clutch and egg size for *Barbarophryne brongersmai* (Hoogmoed, 1972) was also generated de novo for this study, but refers to a laid clutch from a breeding program, not from a dissected female.

Clutch sizes below 500 eggs were counted exactly and clutches larger than this were divided into smaller, equal sized portions, one of which was counted and this number was then multiplied by the number of egg portions to get an estimate total clutch size. Egg diameter was measured to the nearest 0.1 mm using dial callipers. Where possible, multiple individuals of each species were dissected to obtain repeated measures per species.

2.2. Statistical analyses

The ideal dataset for this kind of study would consist of egg, clutch and body size measurements of the same female. However,

this information is rarely published and so maximum records per species were used, which produces the most extensive dataset. Snout-vent length, the measurement from the tip of the snout to the cloaca, was used as a body size measurement, egg diameter without a gelatinous layer was used as an egg size measurement and counts of the number of eggs in one clutch determined clutch size. All measures were natural log transformed, and correlations of egg and clutch size with body size were explored with linear regressions. Separate regression slopes were calculated for species with different reproductive modes and clutch types (ovarian and laid clutches). Reproductive mode categories were defined as development as free-swimming tadpoles (including *Altiphryniodes malcolmi* [Grandison, 1978], which is arguably not strictly free-swimming, but see discussion), lecithotrophic viviparity and matrotrophic viviparity (as defined by Wourms, 1981), however the last was excluded from statistical analyses due to having a sample size of one (*Nimbaphrynoides occidentalis*). All coding is listed in Table 1. To test whether the regression slopes were significantly different for each of the groupings, Analyses of Covariances (ANCOVAs) with type III sum of squares were carried out using the Anova function in the *car* package (Fox and Weisberg, 2011) in R v.3.0.0 (R core team, 2013). In cases where the assumptions for parametric testing were not met, significance was tested using a permutation test implementing the *aovp* function in the R package *lmPerm* (Wheeler, 2010). The residuals for egg and clutch size on body size of a reduced dataset with species containing missing data removed were then used to plot egg size residuals against clutch size residuals. Although the variables at hand show linear relationships (after natural log transformations), using residuals to partial out the effect of a third variable is still considered bad practice (Garcia-Berthou, 2001) and this was therefore only done to graphically explore the relationship between these two traits. To statistically test whether a significant correlation exists and whether this is affected by either reproductive modes or clutch types, ANCOVAs with female body size as a covariate were carried out. For all tests, non-significant interaction terms were removed and if the reduced model was not a significantly worse fit (tested using the anova function in the basic *stat* package in R), this model was preferred.

3. Results

Egg and clutch size data was collected from dissections of 35 females covering 19 species (Table 1S). The total dataset comprises 60 species (of just over 100 described species of African bufonids; AmphibiaWeb, 2013), clutch size data for 56, and egg size data for 54 of these species are included, with 50 species having information for both (Table 1; literature sources in Table S2).

3.1. Clutch size

The frequency distribution of clutch sizes is heavily skewed with the majority of African bufonid species laying less than 2000 eggs per clutch (mean = 3597; Fig. 1A). For the complete data set, clutch size is strongly, positively related to female body size (β = 3.552, adjusted R^2 = 0.818, p < 0.001). When taking account of the different clutch types and reproductive modes, individual regression slopes continue to show a positive relationship of ovarian and laid clutch size with body size (Fig. 1B and C), however this relationship is not statistically supported for lecithotrophic viviparous species (adjusted R^2 = 0.306, p = 0.071).

The ANCOVA on clutch size and body size with clutch type as a treatment effect shows that there is no significant interaction between body size and clutch type suggesting that the two clutch type slopes are similar and the interaction term can be removed

Table 1

Maximum female body size (measured as snout-vent length in mm), clutch size and egg size (diameter in mm) for all species included in this study and coding for the two treatment classes "clutch type" (whether data originated from field observations of laid clutches [laid], or dissected gravid females from museum collections [ovarian]) and "reproductive mode" (whether species undergo larval development as free-swimming tadpoles [FST] or give birth to live young in the form of either lecithotrophic [LV] or matrotrophic viviparity [MV]).

Species	Max. female body size (in mm)	Max. clutch size	Max. egg size (in mm)	Clutch type	Reproductive mode
Altiphrynoides malcolmi (Grandison, 1978)	31	31	3.9	laid	FST
Altiphrynoides osgoodi (Loveridge, 1932)	62	307	3	laid	FST
Amietophrynus brauni (Nieden, 1911)	110	9000	1	ovarian	FST
Amietophrynus camerunensis (Parker, 1936)	91	2100	1.7	ovarian	FST
Amietophrynus channingi Barej, Schmitz, Menegon, Hillers, Hinkel, Böhme and Rödl, 2011	143	4500	2	laid	FST
Amietophrynus funereus (Bocage, 1866)	66	unknown	1.4	ovarian	FST
Amietophrynus garmani (Meek, 1897)	115	20,000	1.2	laid	FST
Amietophrynus gracilipes (Boulenger, 1899)	41	unknown	1.5	laid	FST
Amietophrynus gutturalis (Power, 1927)	120	23,000	1.45	laid	FST
Amietophrynus kisoloensis (Loveridge, 1932)	87	2400	1.9	ovarian	FST
Amietophrynus lemairii (Boulenger, 1901)	70	2500	1.5	ovarian	FST[a]
Amietophrynus maculatus (Hallowell, 1854)	80	8000	1.5	laid	FST
Amietophrynus mauritanicus (Schlegel, 1841)	150	10,000	1.5	laid	FST
Amietophrynus pantherinus (Smith, 1828)	140	24,476	unknown	laid	FST
Amietophrynus pardalis (Hewitt, 1935)	147	14,000	1.5	ovarian	FST
Amietophrynus poweri (Hewitt, 1935)	100	23,000	unknown	laid	FST
Amietophrynus rangeri (Hewitt, 1935)	115	10,760	1.3	laid	FST
Amietophrynus regularis (Reuss, 1833)	130	11,000	1.3	laid	FST
Amietophrynus superciliaris (Boulenger, 1888)	163	4000	2	laid	FST
Amietophrynus tuberosus (Günther, 1858)	74	4200	1.5	ovarian	FST
Amietophrynus xeros (Tandy, Tandy, Keith, and Duff-MacKay, 1976)	92.7	5000	1	laid	FST
Barbarophryne brongersmai (Hoogmoed, 1972)	51	690	1.7	laid	FST
"Bufo" pentoni Anderson, 1893	95	2600	2	laid	FST
Capensibufo rosei (Hewitt, 1926)	39	90	2.5	laid	FST
Capensibufo tradouwi (Hewitt, 1926)	48	60	2	laid	FST
Didynamipus sjostedti Andersson, 1903	19	18	2.3	ovarian	FST[a]
Duttaphrynus dodsoni (Boulenger, 1895)	64	470	1.5	ovarian	FST
Laurentophryne parkeri (Laurent, 1950)	27.1	30	2.0	ovarian	unknown
Mertensophryne anotis (Boulenger, 1907)	46	105	2.5	laid	FST
Mertensophryne howelli (Poynton and Clarke, 1999)	45	60	2.5	ovarian	FST[a]
Mertensophryne lindneri (Mertens, 1955)	34	81	2.1	ovarian	FST[a]
Mertensophryne lonnbergi (Andersson, 1911)	44	125	2.5	laid	FST
Mertensophryne loveridgei (Poynton, 1991)	38	131	2.1	ovarian	FST[a]
Mertensophryne melanopleura (Schmidt and Inger, 1959)	27	35	2	laid	FST
Mertensophryne micranotis (Loveridge, 1925)	24	70	1.8	ovarian	FST
Mertensophryne taitana (Peters, 1878)	33	350	2	laid	FST
Mertensophryne usambarae (Poynton and Clarke, 1999)	45	60	2.4	ovarian	FST[a]
Mertensophryne uzunguensis (Loveridge, 1932)	30	188	2	ovarian	FST
Nectophryne afra Buchholz and Peters, 1875	25	40	2.5	ovarian	FST
Nectophryne batesii Boulenger, 1913	25	45	2.5	ovarian	FST
Nectophrynoides asperginis Poynton, Howell, Clarke and Lovett, 1999	29	16	2.4	laid	LV
Nectophrynoides cryptus Perret, 1971	34	25	2.2	ovarian	LV
Nectophrynoides laticeps (Channing, Menegon, Salvidio and Akker, 2005)	24	60	1.8	ovarian	LV[a]
Nectophrynoides minutus Perret, 1972	22	31	2	ovarian	LV
Nectophrynoides paulae Menegon, Salvidio, Ngalason and Loader, 2007	24	20	unknown	ovarian	LV[a]
Nectophrynoides poyntoni Menegon, Salvidio and Loader, 2004	24	10	unknown	ovarian	LV[a]
Nectophrynoides tornieri (Roux, 1906)	34	37	2	laid	LV
Nectophrynoides vestergaardi Menegon, Salvidio and Loader, 2004	24	46	unknown	ovarian	LV[a]
Nectophrynoides viviparus (Tornier, 1905)	60	160	2.9	ovarian	LV
Nimbaphrynoides occidentalis (Angel, 1943)	32.5	17	0.6	ovarian	MV
Poyntonophrynus dombensis (Bocage, 1895)	40	900	1.8	laid	FST
Poyntonophrynus fenoulheti (Hewitt and Methuen, 1912)	43	2000	1.8	laid	FST
Schismaderma carens (Smith, 1848)	92	2500	2.5	laid	FST
Vandijkophrynus amatolicus (Hewitt, 1925)	37	unknown	2	laid	FST
Vandijkophrynus angusticeps (Smith, 1848)	58	3000	2	laid	FST
Vandijkophrynus gariepensis (Smith, 1848)	95	unknown	1.5	laid	FST
Vandijkophrynus robinsoni (Branch and Braack, 1996)	57	2000	unknown	laid	FST
Werneria bambutensis (Amiet, 1972)	38	483	2	ovarian	FST
Werneria tandyi (Amiet, 1972)	41.2	629	1.5	ovarian	FST
Wolterstorffina parvipalmata (Werner, 1898)	35	2.5	219	laid	FST

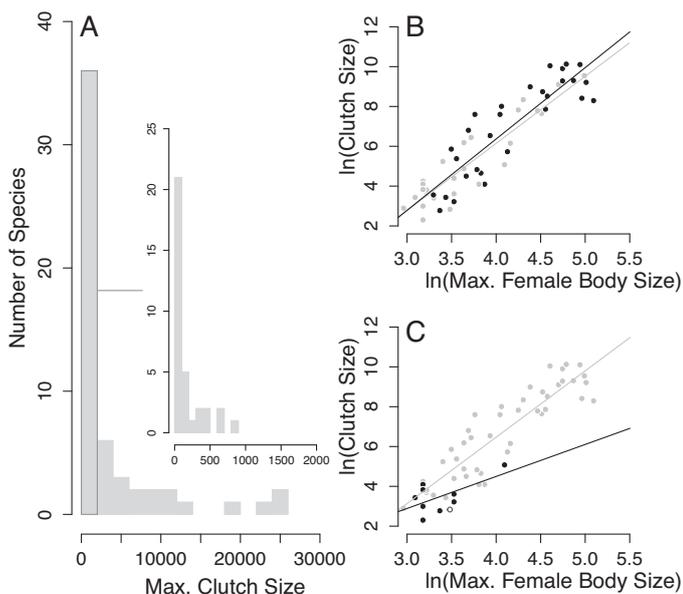Cases where reproductive mode is assumed are indicated with the annotation [a].

Fig. 1. Clutch sizes of African bufonids. (A) Frequency histogram of clutch sizes per species with a magnification of clutch sizes below 2000. (B) Clutch size in relation to female body size with different regression slopes for laid clutches (black; $\beta = 3.583$, adjusted $R^2 = 0.757$, $p < 0.001$) compared to ovarian clutches (grey; $\beta = 3.371$, adjusted $R^2 = 0.817$, $p < 0.001$). (C) Regression slopes for lecithotrophic viviparous species (black; $\beta = 1.607$, adjusted $R^2 = 0.306$, $p = 0.071$) compared to larval developing species (grey; $\beta = 3.331$, adjusted $R^2 = 0.794$, $p < 0.001$). The hollow point represents the matrotrophic viviparous *Nimbaphrynoides occidentalis*.

Table 2a
ANOVA table for effect of body size on clutch size with clutch type as the treatment variable (interaction terms were not significant).

	Sum of Sq.	Df	F	p
Intercept	54.750	1	51.673	<0.001
Female Body Size	210.568	1	198.736	<0.001
Clutch Type	0.487	1	0.460	0.501
Residuals	56.156	53		

from the model. The reduced model is not a significantly worse fit ($F = 0.181$, $p = 0.672$) and is therefore preferred over one including the interaction term. In this model, body size shows a strong, positive effect on clutch size ($F = 198.736$, $p < 0.001$; Table 2a), with no significant treatment effect of clutch type ($F = 0.460$, $p = 0.501$; Table 2a).

The homogeneity of variance assumption of an ANCOVA when using reproductive mode as a treatment effect was not met (Levene's test; $F = 18.817$, $p < 0.001$) and therefore a permutation test was used instead (Table 2b). The interaction term for body size and reproductive mode was not significant and was therefore removed. The reduced model is not a significantly worse fit ($F = 0.2447$, $p = 0.124$) and is therefore preferred over one including the interaction term. For the reduced model, both female body size and reproductive mode were recovered as having a significant effect on clutch size ($F = 179.674$, $p < 0.001$ and $F = 5.676$, $p < 0.05$ respectively; Table 2b), which indicates that although clutch size varies with body size, there is also a difference in pattern between

Table 2b
Permutation ANOVA table for effect of body size on clutch size with reproductive mode as the treatment variable.

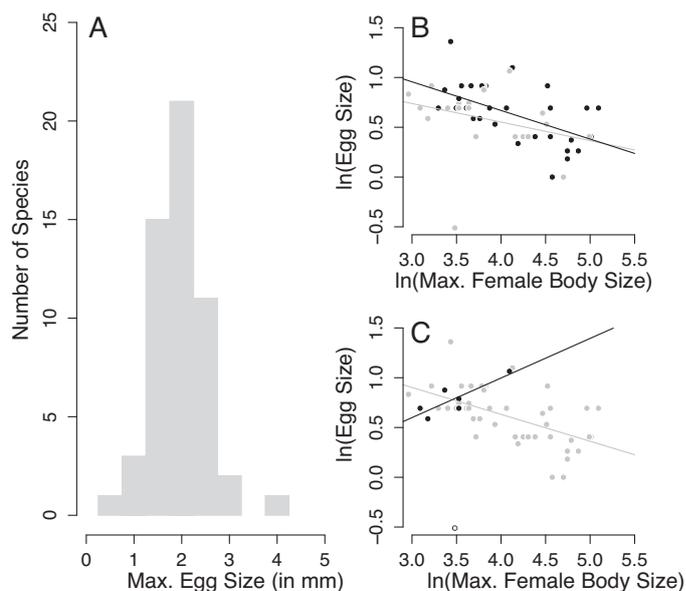|	Df	R Sum of Sq.	R Mean Sq.	F	p
Female Body Size	1	170.387	170.387	179.674	<0.001
Reproductive mode	1	5.383	5.383	5.676	0.021
Residuals	51	48.364	0.948		

Fig. 2. Egg sizes of African bufonids. (A) Frequency histogram of egg size of African bufonids. (B) Egg size in relation to female body size with different regression slopes for laid clutches (black; $\beta = -0.288$, adjusted $R^2 = 0.274$, $p = 0.002$) compared to ovarian clutches (grey; $\beta = -0.133$, adjusted $R^2 = 0.058$, $p = 0.134$). (C) Regression slopes for lecithotrophic viviparous species (black; $\beta = 0.398$, adjusted $R^2 = 0.634$, $p = 0.036$) compared to larval developing species (grey; $\beta = -0.270$, adjusted $R^2 = 0.298$, $p < 0.001$). The hollow point represents the matrotrophic viviparous *Nimbaphrynoides occidentalis*.

lecithotrophic viviparous and free-swimming larva species. The regression slopes depicted in Fig. 1C suggest that for both reproductive modes, a positive relationship of clutch size and female body size can be observed, with the effect being strong in free-swimming larva species, but not statistically different from zero for lecithotrophic viviparous species.

3.2. Egg size

Egg size shows a slight log normal distribution with a mean diameter of 1.936 mm (Fig. 2A). Without sub-setting the data, egg size is inversely correlated to female body size ($\beta = -0.209$, adjusted $R^2 = 0.140$, $p < 0.05$). *Nimbaphrynoides occidentalis*, the only matrotrophic viviparous anuran, is a clear outlier, with an egg size well below what is expected for its body size (represented by a hollow point in Fig. 2C).

The inverse relationship is maintained when sub-setting the data into ovarian and laid clutches, although the slope for ovarian data is not statistically different from zero (adjusted $R^2 = 0.058$, $p = 0.135$; Fig. 2B). If *N. occidentalis* is treated as an outlier and removed, a significant negative relationship is recovered ($\beta = -0.234$, adjusted $R^2 = 0.280$, $p = 0.005$; slope not shown). For reproductive mode as a treatment effect, the regression slope for species with larval development indicates a negative relationship for egg size and body size (adjusted $R^2 = 0.298$, $p < 0.001$; Fig. 2C), but for lecithotrophic viviparous species, this relationship is positive (adjusted $R^2 = 0.634$, $p < 0.05$; Figure 2C).

When comparing the two clutch types, the interaction term for the ANCOVA of egg size and body size is not significant, suggesting that the two slopes are similar and as for clutch size, the reduced model is not a significantly worse fit ($F = 0.507$, $p = 0.480$). Body size has a significant effect on clutch size ($F = 12.027$, $p < 0.05$; Table 3a), with no significant treatment effect of clutch type ($F = 2.347$, $p = 0.132$, Table 3a).

When looking at reproductive mode as the grouping variable, the interaction term was significant ($F = 5.399$, $p < 0.05$; Table 3b),

Table 3a

ANCOVA table for effect of body size on clutch size with clutch type as the treatment variable (interaction terms were not significant).

	Sum of Sq.	Df	F	p
Intercept	2.479	1	31.024	<0.001
Female body size	0.961	1	12.027	0.001
Clutch type	0.188	1	2.347	0.132
Residuals	4.075	51		

Table 3b

ANCOVA table for effect of body size on clutch size with reproductive mode as the treatment variable.

	Sum of Sq.	Df	F	p
Intercept	2.616	1	51.912	<0.001
Female body size (FBS)	1.089	1	21.623	<0.001
Reproductive mode (RM)	0.264	1	5.238	0.027
FBS × RM	0.272	1	5.399	0.024
Residuals	2.418	48		

meaning the slopes of the two regression lines (Fig. 2C) are significantly different from one another. Body size is inversely correlated with egg size in larval developing species but the reverse is true for lecithotrophic viviparous species.

3.3. Egg vs. clutch size

Clutch size and egg size regressions on body size are more or less linear (see Figs. 1 and 2) and therefore the residuals of each regression could be used as a means of removing the effect of body size. When doing so, there is a negative overall relationship between the residuals of egg size and clutch size ($\beta = -0.079$, adjusted $R^2 = 0.064$, $p = 0.045$). This relationship is intensified when *Nimbaphrynoides occidentalis* is removed ($\beta = -0.127$, adjusted $R^2 = 0.308$, $p < 0.001$). For both laid and ovarian clutches, the negative relationship is maintained (Fig. 3A), but only if *N. occidentalis* is removed, is the slope for the ovarian clutch dataset significantly different from zero ($\beta = -0.166$, adjusted $R^2 = 0.459$, $p < 0.001$; regression line not shown). For the regression slopes representing the different reproductive modes, both larval developing and lecithotrophic viviparous species show a negative relationship (Fig. 3B) although the relationship for the latter is not statistically different from zero ($\beta = -0.180$, adjusted $R^2 = 0.230$, $p = 0.189$).

When comparing the two clutch types (not including *N. occidentalis*), none of the interaction terms for the ANCOVA are significant suggesting the slopes are similar and the reduced model is not a significantly worse fit ($F = 0.940$, $p = 0.451$). In the reduced model, clutch size has the strongest effect on egg size ($F = 21.303$, $p < 0.001$; Table 4a) with female body size and clutch type having no significant effect ($F = 2.148$, $p = 0.150$ and $F = 3.864$, $p = 0.056$ respectively; Table 4a).

When comparing the two reproductive modes, again, none of the interaction terms for the ANCOVA are significant and similarly, the reduced model is not a significantly worse fit ($F = 1.219$, $p = 0.318$). In the reduced model, reproductive mode has no significant effect on the model ($F = 2.057$, $p = 0.159$; Table 4b) and

Table 4a

ANCOVA table for effect of clutch size on egg size with female body size and clutch type as covariates (interaction terms were not significant).

	Sum of Sq.	Df	F	p
Intercept	0.356	1	9.734	0.004
Clutch size	0.778	1	21.303	<0.001
Female body size	0.078	1	2.148	0.150
Clutch type	0.141	1	3.864	0.056
Residuals	1.607	44		

Table 4b

ANCOVA table for effect of clutch size on egg size with female body size and reproductive modes as covariates (interaction terms were not significant).

	Sum of Sq.	Df	F	p
Intercept	0.274	1	7.207	0.010
Clutch size	0.852	1	22.454	<0.001
Female body size	0.134	1	3.537	0.067
Reproductive mode	0.078	1	2.057	0.159
Residuals	1.670	44		

the main driver is clutch size ($F = 22.454$, $p < 0.001$; Table 4b) with female body size not contributing significantly ($F = 3.537$, $p = 0.067$; Table 4b).

4. Discussion

In African bufonids, both egg number per clutch and egg size are correlated with body size. As body size increases, clutch size increases, but egg size decreases, and when correcting for body size, a strong negative correlation is evident for egg size on clutch size. Whether data originated from laid clutches or from dissected females had no effect on any general patterns and thus, we propose that data from both sources could be combined for broad-scale comparative studies in the future. However, reproductive mode had a significant effect on how egg size and clutch size are correlated with body size (though not on how these two parameters are correlated with each other after correcting for body size), in line with what Wells (2007) observed for Neotropical species. Our dissections of *Mertensophryne micranotis* (Loveridge, 1925) and *M. uzunguensis* (Loveridge, 1932) also provide new record number of eggs for these species, with egg counts for both exceeding any previous records by a factor of two or more (Grandison and Ashe, 1983; Poynton et al., 2005).

Larval developing species retain a significant, positive correlation of clutch size with body size as well as inverse correlations of egg size with body size, and of egg size with clutch size (after correcting for body size). For lecithotrophic viviparous species, the slopes of the regression lines for clutch size on body size and for body size corrected egg size on clutch size showed the same trends as for larval developing species, however they were not significantly different from zero, suggesting weak correlations. The regression slope for egg size on body size was significantly different from zero and supported a positive correlation of egg size with body size, the reverse for what was recovered for larval developing species.

The positive relationship between clutch size and body size is one that has been recovered in previous studies on amphibians (Kuramoto, 1978; Barbault, 1984; Duellman and Trueb, 1994; Prado and Haddad, 2005; Wells, 2007) and the most straight forward explanation for this is that larger bodied females can carry larger numbers of eggs (Roff, 2002). This however assumes that egg size is relatively constant and one cannot rule out that both body size and fecundity respond to external factors in a collinear fashion and thus there may not be a direct causal link between the two. For example, Lüddecke (2002) found that within a single species, body size increased with altitude as did clutch size, even after the effect of increasing body size was removed.

Salthe and Duellman (1973) note that New World anurans practicing the same reproductive mode show a positive interspecific correlation between egg size and female body size, but when investigating this relationship across multiple reproductive modes, the correlation is inverted. Egg size and body size of African Bufonids appears to behave similarly, showing an overall inverse correlation, but as the dataset is subdivided into distinct reproductive modes, lecithortophic viviparous species show a positive correlation. Larval developing species continue to show a negative correlation,
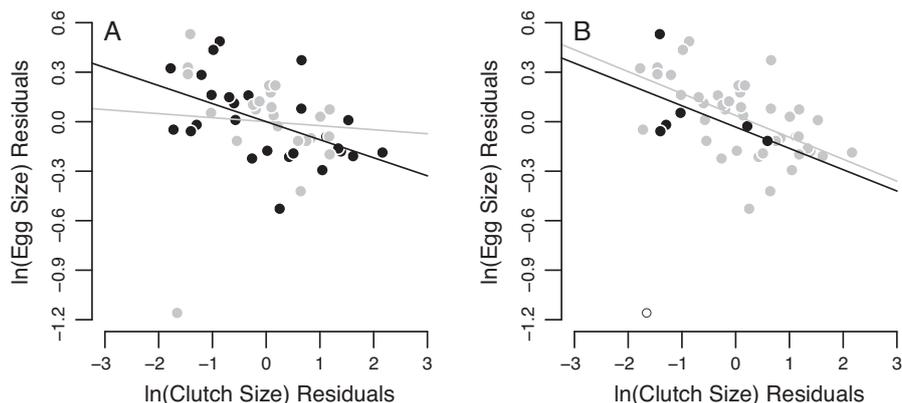
Fig. 3. Residuals for clutch size and egg size on body size, showing different regression slopes for (A) laid clutches (black; $\beta = -0.109$, adjusted $R^2 = 0.224$, $p = 0.009$) and ovarian clutches (grey; $\beta = -0.024$, adjusted $R^2 = -0.043$, $p = 0.757$) and for (B) lecithotrophic viviparous species (black; $\beta = -0.180$, adjusted $R^2 = 0.230$, $p = 0.189$) and larval developing species (grey; $\beta = -0.134$ adjusted $R^2 = 0.327$, $p < 0.001$). The hollow point represents the matrotrophic viviparous *Nimbaphrynoides occidentalis*.

which may be an indication that this category is too heterogeneous, pooling explosive pond breeders such as *Amietophrynus gutturalis* with terrestrial nest breeders such as *Altiphrynoides malcolmi*. Finer categorization of reproductive modes of African bufonids is hampered by the lack of detailed knowledge of the life history of many taxa and highly specialized reproductive modes such as the breeding in tree cavities and provisioning of post-hatching parental care as practiced by *Nectophryne* spp Bucholz and Peters, 1875 are usually represented by too low numbers of species to obtain sufficient sample sizes for statistical testing. A statistically viable refinement of the free-swimming larval developing category would be to firstly remove species with highly specialized modes such as those involving terrestrial nest building (e.g. *Altiphrynoides malcolmi*) or internal fertilization (e.g. *Mertensophryne micranotis*) and then separating species with larva developing in permanent water bodies from those with larva developing in temporary water bodies. The recovered regression slopes for these two groups continue to show negative correlations of egg size and body size ($\beta = -0.178$ and -0.315 respectively), but both slopes are no longer significantly different from zero ($p = 0.063$ and 0.057 respectively).

The viviparous *Nimbaphrynoides occidentalis* produces considerably smaller eggs than is expected for its body size and Angel and Lamotte (1944) comment that the eggs are hugely deprived of yolk. The toad is the only known matrotrophic viviparous anuran and embryos undergo complete development in the uterus of the mother over a period of nine months (Gallien, 1959; Castanet et al., 2000). A similar egg size reduction associated with matrotrophic viviparity has been observed for reptiles (Blackburn et al., 1984) and mammals (Dunbrack and Ramsay, 1989), as developmental energy is no longer provided by yolk stores in the egg, but directly from the mother.

After correcting for body size, bufonids of both reproductive modes (free-swimming larva versus live-bearing) exhibit an inverse correlation between egg size and clutch size, corresponding to previous findings (Duellman and Trueb, 1994; Wells, 2007; Vitt and Caldwell, 2009) as well as the general principle of MacArthur and Wilson's theory of r- versus K-selection in populations (MacArthur and Wilson, 1967; Pianka, 1970). This theory predicts that if there are no density effects or competition, the optimum strategy for an organism would be to maximize fecundity, with minimal investment into each individual (r-selection). If an environment is saturated, the optimum shifts to the other extreme, wherein it is more beneficial for an organism to reduce the number of offspring produced, but to increase the investment per offspring (K-selection). Dobzhansky (1950) reasoned that K-selection should be favoured in climatically stable environments

such as the tropics, whereas in temperate or high altitude regions, r-selection strategies would be more successful. The clear trade-off seen in African bufonids may therefore reflect the environments to which individual species are adapted and therefore offers an interesting system for investigating the relationship of life history parameters and habitat.

The collection of life history data in the field is often difficult. Direct observations of species are often frustrated by the geographical location of species and/or the frequency and rarity of some species. In addition, species with more derived life-histories often breed in cryptic or difficult to observe locations, making it challenging to obtain quantitative and qualitative life history data. This is particularly true for Africa where basic data on the ecology and breeding biology of many species are still lacking. In our study, we compared the utility of data obtained from field observations and museum specimens and proved that there is no significant difference between both data sources, meaning that both sets of data can be combined in more comprehensive analyses. Preserved material from natural history collections is therefore an important resource for significantly adding to our knowledge on amphibian life history.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcz.2014.02.003.

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