Maternal investment increases with altitude in a frog on the Tibetan Plateau

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Abstract

Reproducing females can allocate energy between the production of eggs or offspring of different size or number, both of which can strongly influence fitness. The physical capacity to store developing offspring imposes constraints on maximum clutch volume, but individual females and populations can trade off whether more or fewer eggs or offspring are produced, and their relative sizes. Harsh environments are likely to select for larger egg or offspring size, and many vertebrate populations compensate for this reproductive investment through an increase in female body size. We report a different trade-off in a frog endemic to the Tibetan Plateau, *Rana kukunoris*. Females living at higher altitudes ($n = 11$ populations, 2000–3500 m) produce larger eggs, but without a concomitant increase in female body size or clutch size. The reduced diel and seasonal activity at high altitudes may impose constraints on the maximum body size of adult frogs, by limiting the opportunity for energy accumulation. Simultaneously, producing larger eggs likely helps to increase the rate of embryonic development, causing tadpoles to hatch earlier. The gelatinous matrix surrounding eggs, more of which is produced by large females, may help buffer developing embryos from temperature fluctuations or offer protection from ultraviolet radiation. High-altitude frogs on the Tibetan Plateau employ a reproductive strategy that favours large egg size independent of body size, which is unusual in amphibians. The harsh and unpredictable environmental conditions at high altitudes can thus impose strong and opposing selection pressures on adult and embryonic life stages, both of which can simultaneously influence fitness.

Introduction

The quantity and quality of offspring produced are fundamental life history traits intimately linked with fitness and population viability. Reproductive females have limited energetic resources available to allocate for reproduction, and limited physical space with which to accommodate developing eggs or embryos (Roff, 2002; Castellano *et al.*, 2004). As a consequence, females often trade off the number of offspring produced

with their size (Jørgensen, 1981; Cummins, 1986; Sinervo & Licht, 1991; Lüddecke, 2002; Castellano *et al.*, 2004), which can simultaneously determine the fitness of both mother and offspring (Smith & Fretwell, 1974; Bernardo, 1996; Räsänen *et al.*, 2008). Producing larger eggs or offspring can confer substantial fitness advantages by enhancing survival (Smith & Fretwell, 1974; McGinley *et al.*, 1987; Pike *et al.*, 2008), although in some instances smaller eggs are better (Parichy & Kaplan, 1995). From the mother's perspective, large body size is beneficial for producing more offspring, but is more costly to maintain, especially in environments with limited resources. Local environmental factors often drive the relationships between female body size, egg size, offspring body size and fecundity because of geographical differences in how climate and seasonality

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influence these life stages (Duellman & Trueb, 1986; Morrison & Hero, 2003).

Animals living in high-altitude environments are adapted to harsh environmental conditions (Duellman & Trueb, 1986; Badyaev & Ghalambor, 2001; Oromi *et al.*, 2012). At higher altitudes, many populations experience relatively shorter breeding seasons and activity periods, which is related to longer juvenile stages and larger body sizes at all life stages (Morrison & Hero, 2003). Short breeding seasons and activity periods constrain the opportunity for individual animals to forage, and thus accumulate energy. This is balanced against the consequences of having a large body size, which include a delayed life history and greater energetic requirements (Roff, 2002). These attributes can cause females living at high altitudes to mature later, reproduce less often and produce more clutches of large eggs than smaller conspecifics living at low altitudes (Tilley, 1980; Morrison & Hero, 2003). To date, however, we have a very poor understanding of how maternal investment in reproduction varies among populations of species endemic to high-altitude regions and thus the selective forces driving life history evolution in these range-restricted species.

Amphibians have evolved a wide range of life history strategies to cope with their environments, because of limitations imposed by their ectothermic physiology and vulnerability to desiccation. In high-altitude amphibian populations, growth and development rates are generally slower, breeding seasons are shorter, and females mature later and produce fewer lifetime clutches of larger eggs (Morrison & Hero, 2003). We investigated reproductive trade-offs in the plateau brown frog (*Rana kukunoris*), a species endemic to high-altitude regions of the eastern Tibetan Plateau (2000–4400 m; Xie *et al.*, 2000; Chen *et al.*, 2011). Explosive breeding takes place upon emergence from hibernation in the spring (Lu *et al.*, 2008). After reproducing, females only have a short activity window during which to store energy for the next reproductive bout prior to ceasing activity for the winter (Lu *et al.*, 2008; Chen & Lu, 2011; Chen *et al.*, 2011, 2013). Female plateau brown frogs are the larger sex, and clutch size is positively correlated with female body size (Lu *et al.*, 2008; Chen & Lu, 2011; Chen *et al.*, 2011). Although larger body size allows for the production of relatively more offspring, being large also increases maintenance costs. Thus, having a large body size could be costly at higher altitudes, where shorter activity seasons restrict the amount of energy that an individual can accumulate and store prior to overwintering (Chen *et al.*, 2013). These ecological constraints led us to predict that, in plateau brown frogs, altitude would be (1) negatively associated with clutch size, (2) positively associated with egg size (potentially decreasing the time until hatching into a tadpole and increasing tadpole body size) and (3) positively associated with female body size. Such trade-offs could help balance selection for and against large size in the adult and egg stages.

Materials and methods

We studied maternal investment in 11 *R. kukunoris* populations spanning a 255-km latitudinal gradient along the eastern Tibetan Plateau. Our study sites ranged from 2035 to 3494 m above sea level; we focused our efforts on the higher altitudes (breeding populations below 2900 m are uncommon; Table 1). At breeding ponds, we searched for freshly laid egg clutches. We counted the number of eggs in a small sample of each clutch (ca. 5 g), which was weighed (to 0.01 g) and used to estimate clutch size based on mass of the entire clutch. We quantified egg size by measuring 10–30 eggs from digital photographs of each clutch

Table 1 Study site details, including altitude, latitude and sample sizes and clutch attributes for 11 high-altitude population of *Rana kukunoris* in Tibet. Our study took place from February to April 2013. Note that reproducing populations that are below 2900 m are uncommon.

Altitude (m)	Latitude (degrees)	Number of clutches (n)	Clutch size (SD)	Egg size (SD) [mm]	Clutch volume (SD) [mm³]	Mean body size (SD)	Number of females (n)
2035	33.16	37	733 (3)	2.0 (0.1)	784.9 (247.3)	5.1 (0.5)	17
2597	34.05	6	550 (65)	2.1 (0.1)	678.5 (130.3)	–	–
2926	34.96	6	920 (209)	2.1 (0.0)	1041.5 (231.8)	–	–
3061	32.78	21	815 (254)	2.2 (0.2)	1060.9 (442.3)	5.2 (0.8)	2
3080	34.80	10	760 (294)	2.3 (0.1)	1232.5 (506.5)	–	–
3098	34.77	23	624 (195)	2.2 (0.0)	880.3 (282.0)	5.1 (0.7)	7
3189	34.10	10	1014 (339)	2.2 (0.2)	1299.3 (555.5)	–	–
3462	34.82	172	664 (233)	2.0 (0.1)	640.5 (250.2)	4.8 (0.6)	59
3479	33.89	14	971 (169)	2.0 (0.1)	1014.7 (170.3)	–	–
3485	33.56	5	770 (95)	2.3 (0.0)	1096.2 (135.6)	–	–
3493	34.30	127	740 (210)	2.3 (0.1)	1128.3 (340.1)	5.6 (0.4)	44
Total		431					129

(Abramoff *et al.*, 2004). To obtain a measure of individual egg size, we averaged the minimum and maximum diameter of each egg (to 0.01 mm). All clutches were sampled and measured prior to eggs reaching Gosner Stage 12 (Gosner, 1960) to ensure that egg size measurements were comparable within and among populations (Räsänen *et al.*, 2008).

From a subset of five ponds, we obtained data on maternal investment directly from individual females, which allowed us to understand how maternal body size directly influences clutch size, clutch volume and egg size. To do this, we placed pairs of frogs found in amplexus into buckets of pond water until the female oviposited (< 10 h). Immediately following oviposition, we measured female body size (snout–vent length, SVL to 1 mm), clutch mass, estimated clutch size and took photographs to quantify egg size. After collecting these data, we returned all egg clutches and frogs to their pond of origin.

Life history traits can respond to environmental effects associated with both altitude and latitude, but generally respond more strongly over short spatial scales to decreased annual activity periods caused by altitudinal effects (Oromi *et al.*, 2012). Although we were mainly interested in altitudinal effects on life history traits, we incorporated altitude, latitude and their interaction into our statistical models to help explain more variance in reproductive characteristics among populations. We explored the relationships between environmental variables (altitude, latitude and their interaction) and clutch attributes (clutch size, egg size and clutch volume) using generalized linear mixed models (GLMMs). We treated population as a random variable, altitude and latitude as covariates and clutch parameters as dependent variables. We also tested for trade-offs between egg size and clutch size by first using one of these variables as the dependent variable and the other as the independent variable, and then *vice versa*. Finally, we tested whether female body size influences life history trade-offs both within and among populations differing in altitude by using female body size as a covariate. Prior to analyses, we $\log_{10}$-transformed all variables to better attain normality and enhance homogeneity of variance. To account for the complex covariance structure in our mixed models, we used REML estimation for random effects and the Satterthwaite procedure to approximate degrees of freedom (IBM SPSS Statistics for Windows, Version 20.0; IBM Corp, Armonk, NY, USA).

Results

Clutch size was not significantly associated with altitude ($F_{1,11.5} = 2.666$, $P = 0.130$), latitude ($F_{1,13.3} = 2.3$, $P = 0.153$) or the interaction between these two variables ($F_{1,11.8} = 2.647$, $P = 0.130$). Mean egg size and clutch volume increased significantly with both altitude

(egg size, $F_{1,18.6} = 5.078$, $P = 0.036$; clutch volume, $F_{1,16.9} = 7.475$, $P = 0.014$) and latitude (egg size, $F_{1,22.5} = 5.714$, $P = 0.026$; clutch volume, $F_{1,19.8} = 7.239$, $P = 0.014$) and by the interactions of these two variables (egg size, $F_{1,19.6} = 5.18$, $P = 0.034$; clutch volume, $F_{1,17.5} = 7.466$, $P = 0.014$; Fig. 1). Overall, when accounting for the simultaneous effects of increasing altitude and latitude, egg size and clutch volume both increased (Fig. 1). Breeding populations of this species are uncommon below 2900 m, which restricted our sampling; however, clutch volume remains significant even after removing the lowest two populations ($P < 0.05$). We found no evidence for population-level trade-offs between fecundity and egg size, as evidenced by nonsignificant effects of clutch size on egg size (and *vice versa*; clutch size, $F_{1,421.9} = 1.102$, $P = 0.294$; egg size, $F_{1,321.5} = 0.772$, $P = 0.38$).

We found no significant effect of altitude or latitude, or their interaction, on female body size (all $P > 0.83$). Within populations, however, larger individual females produce significantly heavier clutches ($F_{1,122.9} = 74.674$, $P < 0.001$) containing more eggs ($F_{1,57} = 85.129$, $P < 0.001$), but without a concomitant trade-off with egg size ($F_{1,123.1} = 0.611$, $P = 0.436$). Thus, females at higher elevations and latitudes produce larger eggs and larger clutch volumes than females at lower elevations, but
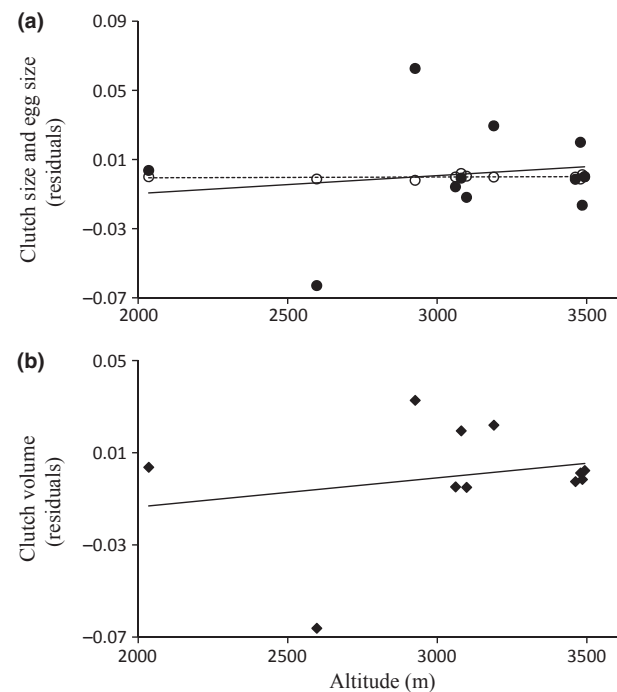
Fig. 1 Relationship between altitude and (a) egg size (solid circles) and clutch size (open circles) and (b) total clutch volume in 11 *Rana kukunoris* populations. Each dot represents the residual mean value for a given population corrected for the effect of altitude, latitude and their interactions. Note that reproducing populations are uncommon below 2900 m.

without a significant increase in body size; thus, body size does not explain the pattern of increased egg size with altitude and/or latitude.

Discussion

Environmental conditions can impose strong selection on life history traits, resulting in a wide range of evolutionary responses by populations living at high altitudes. Many amphibians have responded to these conditions by evolving larger body size, which coevolves with slow life histories and increased reproductive output (Morrison & Hero, 2003). On the Tibetan Plateau, however, the endemic plateau brown frog has evolved a different reproductive strategy. Females living at higher altitudes and latitudes produce larger eggs than females living at lower altitudes and latitudes, but without a concomitant increase in body size. Females from higher-altitude and higher-latitude populations therefore invest relatively more energy into their current breeding and less into longer-term growth and maintenance than females living at lower altitudes (Chen *et al.*, 2013). Shorter seasonal activity periods, combined with physiological constraints on prey digestion, may constrain body size such that larger individuals may be unable to both recover the energy spent on reproduction and store the energy necessary to survive the coming winter. These population-level differences in life history directly influence population demography, which can substantially impact population stability and recovery (Roff, 2002; Morrison & Hero, 2003).

In many amphibian populations, females devote more energy to reproduction with increasing age (Jørgensen, 1992; Czarnoleski & Kozlowski, 1998), resulting in body size-specific reproductive outputs (Salthe & Duellman, 1973; Kuramoto, 1978). Being large increases fecundity and decreases relative energetic investment in reproduction, which speeds recovery of body condition for the next breeding event (Morrison & Hero, 2003). Within populations, larger female *R. kukunoris* produce significantly larger clutch sizes and volumes, but those individuals living at high altitudes were not overly large. This suggests that high-altitude and latitude environments impose constraints on maximum body size, likely because short activity seasons constrain the ability of large individuals to accumulate energy (Chen *et al.*, 2011, 2013). Predation pressure (Kuramoto, 1978; Berven, 1990), interspecific competition (Morrison & Hero, 2003; Mougi & Nishimura, 2007) and survival probability (Travis *et al.*, 1985) can influence life history strategies. In high-altitude populations, there may be lower predation pressure, but also stronger interspecific competition, and/or a lower probability of survival. These pressures may be sufficiently strong to encourage females living at high altitudes to allocate more energy to current breeding efforts, whereas females living at lower altitudes invest more energy into long-term growth, maintenance and survival. Body condition can influence overwintering mortality (Bodie & Semlitsch, 2000; Wells, 2007), and thus, females must be able to store enough energy after reproducing to survive the harsh and extended winter, which may become more difficult for larger individuals.

Trade-offs between clutch size and egg size can influence the fitness of female parents and their offspring (Berven, 1990). Species producing large eggs often lay fewer of them to compensate for increased energy expenditure (Pettus & Angleton, 1967; Crump, 1984), whereas species that lay small eggs tend to have high fecundity (Kuramoto, 1978). Although laying smaller eggs increases fecundity (Crump, 1984), large eggs provide substantial advantages, including larger initial size, faster embryonic development and thus earlier hatching into tadpoles, and earlier metamorphosis into froglets (Kuramoto, 1978; Crump, 1984; Kaplan & King, 1997). The energetic advantage for larger embryos comes in the form of relatively larger yolk sacs containing more nutrients (Komoroski *et al.*, 1998). Despite these potential life history advantages, trade-offs between clutch size and egg size are generally rare in amphibians (e.g. this study; Castellano *et al.*, 2004; Dziminski & Alford, 2005; Laugen *et al.*, 2002; Tejado, 1992). The limited capacity to physically store a clutch comprised of eggs and the associated gelatinous matrix may restrict these trade-offs (Jørgensen, 1981), unless other more proximate factors have stronger influence on maximum body size (Kaplan & Salthe, 1979).

In many extreme environments, life history favours large body size that can accommodate larger clutch volumes and produce more eggs (Pettus & Angleton, 1967; Tilley, 1980; Berven, 1982; Cummins, 1986). If amphibian life history typically favours energetic investment in the number, rather than the size, of eggs (e.g. Jørgensen, 1981), then why does *R. kukunoris* show a different pattern? The especially harsh environment of the Tibetan Plateau may impose unusually strong constraints on maximum body size in frogs, which are susceptible to both temperature and moisture; this could simultaneously favour large eggs, which presumably leads to faster metamorphosis into froglets (Morrison & Hero, 2003; Wells, 2007), and increased gelatinous matrix protecting the eggs, which could help buffer the embryos from temperature extremes (Beattie, 1980; Waldman, 1982).

Populations living in different environments are often adapted to local environmental conditions, which can vary substantially across a species range (Badyaev & Ghalambor, 2001; Oromi *et al.*, 2012). Different environmental pressures will lead to geographical variation in life history traits aimed at maximizing fitness (Lüddecke, 2002; Morrison & Hero, 2003). Harsh high-altitude and high-latitude environments can result in opposing selection on sizes of the embryonic and adult stages. On the Tibetan Plateau, it appears as though

large eggs and female body size are traits that enhance fitness.

Acknowledgments

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