A maladaptive parental effect: offspring survival decreases with maternal over-condition in an amphibian

EMILY A. HARMON[*,ⓞ], TIANXIU LI[†], PATRICK W. KELLY[‡], CATHERINE CHEN[§], DAVID W. PFENNIG[*,ⓞ] and KARIN S. PFENNIG[*]

Department of Biology, CB #3280, University of North Carolina, Chapel Hill, NC 27599, USA
[†]*Current address: Harvard T. H. Chan School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA*
[‡]*Current address: Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695, USA*
[§]*Current Address: Research Development, Knowledge Enterprise, Arizona State University, Tempe, AZ 85287, USA*

Parental effects are often considered an evolved response, in which parents transmit information about the environment to enhance offspring fitness. However, these effects need not be adaptive. Here, we provide a striking example by presenting evidence that overfeeding of adult Mexican spadefoot toads, *Spea multiplicata*, is associated with decreased offspring survival. After a temporary change to their standard feeding regimen, *S. multiplicata* in our captive colony developed a much higher body condition (i.e. body mass for a given body length) than those in the wild. We analysed data from three subsequent experiments and found that although the body condition of a father was positively correlated with tadpole survival, mothers with a higher condition had lower tadpole survival. Our study highlights how obesity can negatively impact future generations via maladaptive maternal effects. Such effects could be especially likely for animals living in variable environments (such as spadefoots) that have evolved 'thrifty phenotypes' that make them prone to obesity. Our study also illustrates how husbandry conditions typically regarded as beneficial might be harmful. Given that captive breeding programmes are increasingly used to combat worldwide amphibian declines, these programmes must consider the ecology and evolutionary history of the focal species to minimize any maladaptive parental effects.

ADDITIONAL KEYWORDS: amphibians – captive breeding – obesity – parental effects – thrifty phenotype hypothesis.

INTRODUCTION

At least 40% of amphibian species are threatened with extinction (e.g. Cox *et al.*, 2022). One approach for saving amphibians involves captive breeding. When coupled with the eventual reintroduction of laboratory-bred animals into natural habitats, captive breeding could prove an important conservation strategy (Rahbek, 1993; Gascon *et al.*, 2007; Ananjeva *et al.*, 2015). However, many challenges with amphibian captive breeding remain, including the management of nutrition, disease and reproductive success (e.g.

Densmore & Green, 2007; Ferrie *et al.*, 2014; Pessier, 2014; Passos *et al.*, 2018). Common failures of captive breeding programmes include an inability to initiate reproductive behaviour, low fecundity rates and low offspring survival (Browne & Zippel, 2007; Kouba & Vance, 2009; Silla *et al.*, 2021).

Breeding success depends not only on the treatment of offspring, but also on the health and physiological state of their parents. Adult body condition, or energy reserves in the body (Peig & Green, 2009), can influence offspring phenotypes through parental effects (e.g. Bennett & Murray, 2014). For example, in many amphibians, maternal effects arise when mothers allocate resources differentially to eggs, which can affect the success of their offspring (Kaplan, 1998).

[*]Corresponding authors. E-mail: eaharmon@live.unc.edu; dpfennig@unc.edu; kpfennig@unc.edu

1

Because of this link between maternal body condition, egg size and offspring traits, females in better condition are generally assumed to produce more and higher-quality offspring (Salthe & Duellman, 1973; Kaplan & Salthe, 1979; Kaplan, 1987; Kaplan & King, 1997; Okamiya *et al.*, 2021). Indeed, in many species (not only amphibians), bigger females can produce higher-quality offspring (Steiger, 2013). Likewise, paternal body condition is often associated with offspring success. For example, male body size or condition can be positively associated with higher offspring fitness as measured by longer life expectancy, increased growth rate or better competitive ability (i.e. a 'good genes' effect; reviewed by Rosenthal, 2017; e.g. Welch *et al.*, 1998; Pfennig, 2008; Kelly *et al.*, 2019, 2021).

Given the threatened status of amphibians and the aforementioned evidence that high parental body condition increases offspring success, it makes sense to boost the body condition of breeding adults in captive colonies (e.g. Jayson *et al.*, 2018). Yet, such a strategy might become detrimental when feeding adults beyond the natural levels for which a given species or population has evolved. An extreme mass for a given size could imply a disease like obesity rather than good body condition (Peig & Green, 2009). Indeed, captive amphibians are often obese owing to calorie excess and inactivity (McWilliams, 2008). Therefore, increasingly higher parental condition or mass for body size might not always increase fitness. For instance, amphibians exposed to high levels of dietary fat for a prolonged period could develop corneal lipidosis, which would interfere with hunting prey (Shilton *et al.*, 2001; Keller & Shilton, 2002; McWilliams, 2008).

Recent observations in our captive colony of Mexican spadefoot toads, *Spea multiplicata*, suggest that the 'bigger is better' strategy is potentially problematic. In particular, several breeding events for various laboratory experiments yielded low tadpole survival compared with previous laboratory breeding events, despite troubleshooting the conditions in which tadpoles were reared. Across these failed breeding events, we noted that the adults bred in our experiments had higher mass for body size than those used previously. Indeed, the adults appeared obese relative to previously bred toads and those in the wild. Adult and tadpole husbandry protocols, used successfully for > 15 years, had not changed substantially except for the frequency and amount of adult feeding, which had increased. We therefore suspected a previously underappreciated parental effect in amphibians: adult obesity might have contributed to reduced offspring fitness. We treated the change in adult mass for body size (i.e. the mass of an individual relative to its body length) as an unplanned experiment to evaluate the effects of parental mass for body size on offspring survival.

Here, we provide evidence that in *S. multiplicata*, parental mass for body size predicted tadpole survival in a counter-intuitive manner: as female mass for body size increased, the survival of their tadpoles decreased. Our study is noteworthy because few studies discuss the ramifications of overly high mass for body size in amphibians and how it might negatively impact reproduction. Such a pattern could be especially likely to arise in species and populations (such as spadefoots) with natural and evolutionary histories that make them prone to obesity. Our data suggest the need for further research on the subject, including the mechanistic basis of such maladaptive parental effects. Understanding different risk factors for the health and reproduction of captive animals, including the ecology and evolutionary history of the focal species, is essential for the improvement and ultimate success of captive breeding programmes.

MATERIAL AND METHODS

DATA SOURCES

We have bred wild-caught spadefoot toads successfully in our laboratory at University of North Carolina, Chapel Hill for > 15 years (e.g. Pfennig & Rice, 2007; Pfennig *et al.*, 2007; Ledon-Rettig *et al.*, 2008; Pfennig & Martin, 2009; Levis *et al.*, 2015, 2017b; Kelly *et al.*, 2019; Seidl *et al.*, 2019; Chen & Pfennig, 2020). However, beginning in 2019 and following a colony-wide increase in adult feeding, we noted unusually high levels of tadpole mortality from planned breeding events. To investigate this pattern, we compiled data opportunistically on parent mass for body size and offspring survival from three experiments conducted in 2019, 2020 and 2021 for other purposes.

We collected all adult spadefoot toads from natural populations near Portal (Arizona [AZ], USA) and maintained them in our laboratory at the University of North Carolina, Chapel Hill. One to three animals were kept in 37 cm × 22 cm × 24.5 cm inch Kritter Keeper boxes filled halfway with moist play sand. The boxes were kept on racks in rooms with a 14 h light–10 h dark cycle and kept in a temperature range of 23.9–26.7 °C and air humidity range of 30–85% (although substrate was always moist). Every other week, spadefoots foraging on the surface were fed approximately two crickets dusted with Rep-Cal herptivite multivitamins and calcium powder with vitamin D_3. On alternate weeks, all spadefoots were dug up and fed approximately four crickets. Crickets were maintained on Fluker's orange cube diet and 'gut-loaded' with Fluker's high calcium diet. We also dusted the crickets with nutrient powder to increase their content of protein, fat, fibre, calcium, phosphorus

and other vitamins. These general protocols have been used successfully to maintain *S. multiplicata* from these populations near Portal, AZ, in addition to other spadefoot toad species, for > 15 years. However, before 2019, adults were given approximately one or two crickets per feeding. The increased numbers of crickets as above were implemented by university animal care staff attempting to increase mass for body size according to the visual score described for the mountain chicken frog, *Leptodactylus fallax* (Jayson *et al.*, 2018), which

is in a different family (Leptodactylidae) from that of spadefoots (family Scaphiopodidae). Under this feeding regimen, the mean mass for body size of *S. multiplicata* in our colony increased substantially (Fig. 1).

For each breeding, we paired females with males from the same or nearby populations. We induced breeding by injecting each adult with 0.07 mL of 0.1 mM luteinizing hormone-releasing hormone, placing each pair of adults in a 20 L tank filled with dechlorinated tap water, and leaving them undisturbed overnight.
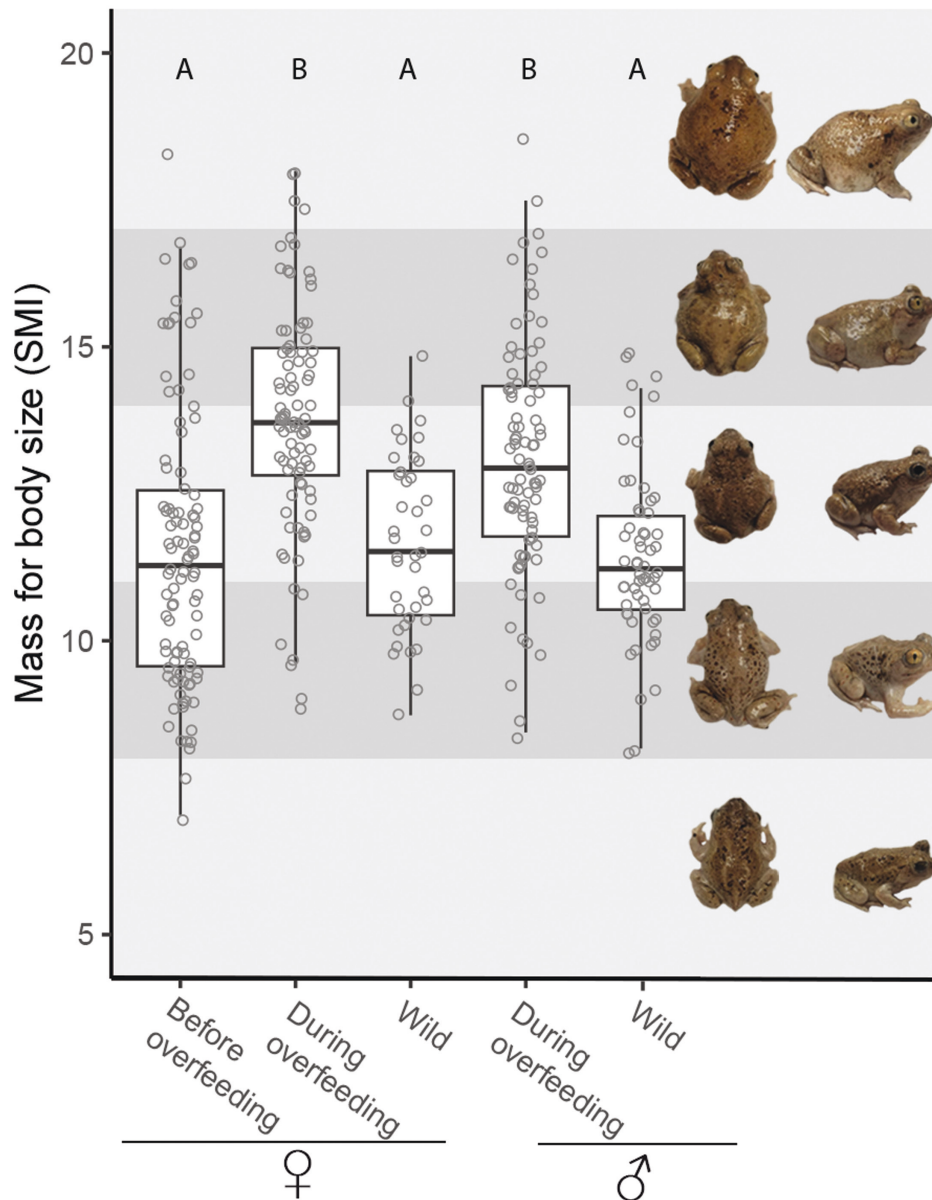

Figure 1. Mass for body size [measured as scaled mass index (SMI)] of *Spea multiplicata* in the laboratory colony before the increase in feeding and during the period of overfeeding, and for adults from the same populations in the wild. Each point represents an individual spadefoot measured, and the box and whisker plots show the quartiles of each group. Different letters at the top denote statistically distinct groups. Photographs illustrate the appearance of individuals falling within a shaded scaled mass index range.

© 2023 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2023, **XX**, 1–11

We returned adults to the colony in the morning and aerated clutches (*Spea* produce ~1000 eggs) until they hatched ~48 h later. We tested the source water daily and adjusted the pH to 7 if needed using Proper pH 7.0. Although *S. multiplicata* are tolerant of ammonia levels of ≥5 mg/L in nature (Sandoz *et al.*, 2017), we spot checked pH, nitrate, nitrite and ammonia in individual boxes throughout the experiments and performed partial water changes at least every other day or if water became cloudy. Upon completing the three experiments described below, we euthanized tadpoles or metamorphs via submersion in tricaine methanesulfonate (MS-222). All of these procedures (conducted under IACUC protocols 20-216.0 and 20-036.0) were similar to those used previously to breed spadefoot toads successfully (e.g. Pfennig & Rice, 2007; Pfennig *et al.*, 2007; Ledon-Rettig *et al.*, 2008; Pfennig & Martin, 2009; Levis *et al.*, 2015, 2017b; Kelly *et al.*, 2019; Seidl *et al.*, 2019; Chen & Pfennig, 2020).

SPECIFICS OF THE BREEDING EVENTS

For the 2019 experiment, we bred 30 male *S. multiplicata* that had been collected the previous summer with females from the same population (the females had been maintained in captivity for ≤ 3 years). From each of the 20 largest clutches, we removed and isolated 24 or 25 eggs in plastic Solo cups containing 300 mL of dechlorinated tap water. We fed the resulting tadpoles equal amounts of fish food and live-cultured brine shrimp until they reached metamorphosis, ending the experiment after 7 weeks. Data obtained from this experiment included parental mass for body size and collection year, and the time to death for each tadpole that died spontaneously.

For the 2020 experiment, we used data from 28 pairs of *S. multiplicata*, all of which had been held in captivity for 1– 4 years. Of the 28 pairs, 27 produced eggs and 25 produced hatchlings. When their tadpoles reached the free-swimming stage, we raised ≤ 40 tadpoles per family singly in 111 mm × 111 mm × 115 mm boxes filled with 900 mL of dechlorinated tap water. We fed tadpoles fish food *ad libitum* for 14 days. Data obtained from this experiment included parental mass for body size and collection year, and whether each singly raised tadpole survived for 14 days.

The 2021 experiment evaluated whether dosing the mothers with vitamin A would improve tadpole survival rates. We chose 30 *S. multiplicata* females to assign haphazardly to two groups containing females of various mass for body size and collection years (3–12 years in captivity). We fed the control group (N = 15) their typical diet, which is likely to contain undetectable levels of vitamin A (Finke, 2015), and fed the vitamin A treatment group (N = 15) a dose of crickets dusted with Repashy vitamin A supplement

weekly for 4 weeks. We then bred the females with males (2–10 years in captivity) from the same or nearby populations. Of the 30 pairs, 26 produced eggs and, of these, 20 produced hatchlings. We counted the proportion of eggs that hatched by photographing each family before and after hatching. When hatchlings reached the free-swimming stage (1 day post-hatching), we gently divided the clutches into groups of 30–40 hatchling tadpoles per litre (families were housed separately). These groups were then placed in 375 mm × 257 mm × 143 mm boxes filled with 8.5 L of dechlorinated tap water. We fed tadpoles *ad libitum* fish food and, starting at 5 days post-hatching, live-cultured brine shrimp. We removed dead tadpoles and conducted partial water changes daily. To track survival, we photographed every container of tadpoles daily for 14 days. Data obtained from this experiment included the parental mass for body size and collection year, and daily counts of the number of living tadpoles in each family. All data are available in the Supporting Information (Tables S1, S2).

EVALUATION OF MASS FOR BODY SIZE

To calculate mass for body size, we used Peig & Green's (2009) scaled mass index because it is a reliable, non-destructive method that outperforms other condition indices and performs well for the tested amphibians (Peig & Green, 2009, 2010; MacCracken & Stebbings, 2012). We calculated the mass for body size for each adult as: $\hat{M}_i = M_i(L_0/L_i)^{b_{SMA}}$, where M_i and L_i are the body mass and the snout–vent length of individual i, respectively; b_{SMA} is the scaling exponent estimated by the standardized major axis (SMA) regression of $\ln(M)$ on $\ln(L)$; L_0 is the arithmetic mean value of L; and $\hat{M}_i$ is the predicted body mass for individual i when the linear body measure is standardized to L_0 (as done by Chen & Pfennig, 2020; Kelly *et al.*, 2021). We compiled a reference dataset to compute common values of L_0 and b_{SMA}, containing 418 mass and snout–vent length measurements of *S. multiplicata* taken in the wild between 1998 and 2019 (Supporting Information Table S3). Note that our measures of scaled mass index from the field were likely to be biased towards higher values, because we collected only adults that appeared healthy and in good condition. Therefore, these measurements represent a range of natural mass for body size for healthy *S. multiplicata* from populations near Portal, AZ, USA.

We first evaluated whether the scaled mass index of adults in our colony increased with the feeding regimen and was (as we suspected) higher than that of adults in the wild. To do so, we compared the scaled mass index measures for: *S. multiplicata* males and females used in the three experiments that had all been measured at the time of the experiment; *S.*

multiplicata females residing in our colony that had been measured the year before the change in feeding (Supporting Information Table S4); and field-collected *S. multiplicata* males and females from the same populations that were measured at time of collection. The data were normally distributed but unbalanced; therefore, we performed a type III ANOVA test followed by Tukey's honestly significant difference (HSD) post hoc test to compare the scaled mass index of male and female *S. multiplicata* in captivity with each other and with those in the wild, using the 'car' package in R.

We also examined the effects of how long parents had been in captivity. We did so because the adults bred between 2019 and 2022 had been in captivity longer than those typically used for our experiments (because of our inability to replenish our laboratory colony with new animals owing to drought at our field sites and travel limitations during the coronavirus disease 2019 pandemic). We hypothesized that mass for body size increases with time in captivity. We therefore used a linear mixed-effects model to assess the relationship between mass for body size and years in captivity by sex, with collection year and population as random intercepts, using the 'lme4' and 'lmerTest' packages in R. This model was overfitted for males; therefore, males were analysed by linear regression without random effects. We confirmed that the data met model assumptions, including normality of residuals, via a Shapiro–Wilk test.

SURVIVAL ANALYSIS

We used IMAGEJ to count the number of eggs, hatchlings and tadpoles from each photograph taken during the 2021 experiment. Daily counts from the 2021 experiment were used to record the status (alive or dead) of each tadpole at 14 days and its date of death, if applicable. Given that our short-term vitamin A supplementation of mothers had no impact on tadpole survival ($P = 0.660$), we combined the 2019 and 2021 datasets. We thereby compiled a dataset of individual survival/time of spontaneous death events over 14 days for 18 714 tadpoles across 45 clutches. To evaluate the relationship between offspring survival, on the one hand, and maternal and paternal mass for body size and time in captivity, on the other hand, we performed survival analyses using Cox mixed-effects models fitted by maximum likelihood using the 'coxme' package in R. We set the maternal and paternal mass for body size and number of years in captivity as fixed effects and the family identity nested within the source experiment as random intercepts.

Separately, we evaluated the relationship between offspring survival and parental factors using data from the 2020 experiment. From data listing the number of tadpoles that survived throughout the 14 day duration of the experiment, we created a dataset listing survival/death for each tadpole. We then performed a logistic regression in a generalized linear mixed-effects model, which does not require normally distributed errors of the response variables, using the 'lme4' package in R. We set tadpole survival/death as the response, maternal and paternal mass for body size and years in captivity as fixed effects, and family as a random effect. We added a quadratic term to the model to test for linearity of the relationships and determine whether it improved the model according to an *F* test. The quadratic term was not significant for any variable. We checked for collinearity between variables, revealing low collinearity for all variables (variance inflation factor < 1.5) and used Wald χ^2 tests for inference using the 'car' package in R (R Core Team, 2018).

RESULTS

Before the change in feeding, mass for body size (measured as the scaled mass index) was similar between animals in the colony and the wild ($P = 0.992$; Fig. 1). As expected, after the new feeding regimen began, the mass for body size of male and female *S. multiplicata* in our colony was higher than that of their wild counterparts ($F_{4,362} = 23.257$, $P \ll 0.001$). The sexes did not vary in mass for body size in captivity ($P = 0.177$) or in the wild ($P = 0.982$). We also found a positive correlation between mass for body size and time in captivity for males ($R^2 = 0.0858$, $F_{1,85} = 9.07$, $P = 0.00342$) but not for females (Kenward–Roger *F*-test; $R^2 = 0.0592$, $F_{1,18} = 2.391$, $P = 0.140$; Fig. 2).

For the combined 2019 and 2021 datasets, parental mass for body size and maternal years in captivity predicted offspring survival. Specifically, for every one unit increase in paternal mass for body size, the risk of tadpole death decreased by 22.68% ($Z = -2.23$, $P = 0.026$). In contrast, for every one unit increase in maternal mass for body size, the risk of tadpole death increased by 31.58% ($Z = 2.20$, $P = 0.028$). Furthermore, for each additional maternal year in captivity, the risk of tadpole death increased by 28.22% ($Z = 2.15$, $P = 0.032$). In contrast, paternal time in captivity did not impact offspring mortality risk ($Z = 1.16$, $P = 0.250$), although it should be noted that all males used in the 2019 experiment were captured in the same year (albeit ages at the time of capture are unknown).

Data from the 2020 experiment also showed that maternal mass for body size impacted offspring survival. Specifically, tadpoles of mothers with higher mass for body size were again less likely to survive ($\chi^2_1 = 7.101$, $P = 0.00771$); paternal mass for body size, in contrast, did not impact tadpole survival
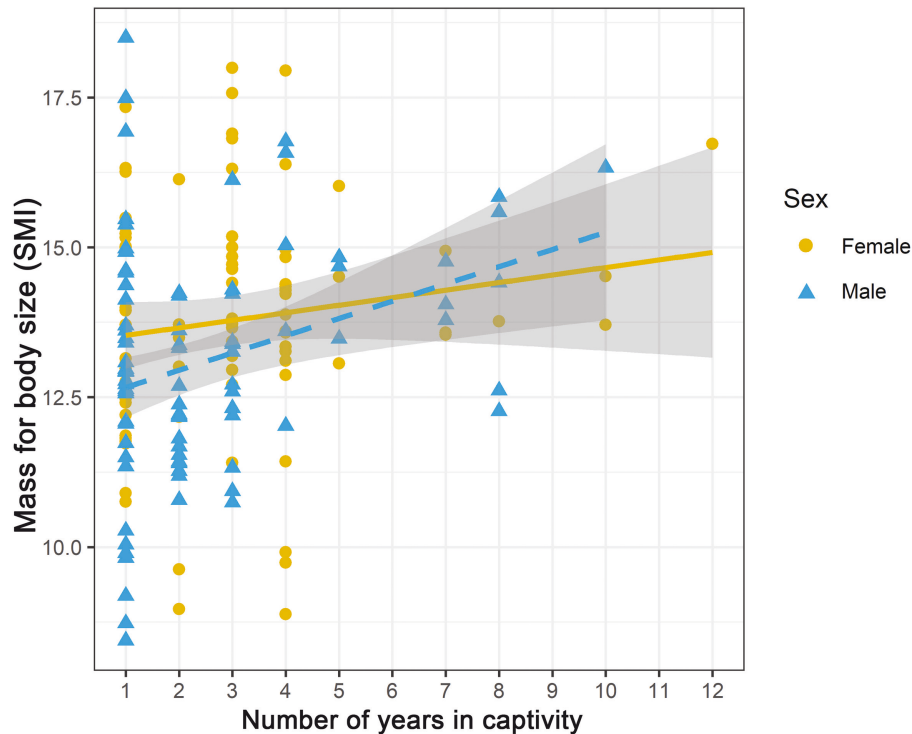
Figure 2. Mass for body size [measured as scaled mass index (SMI)] and years spent in captivity at the time of breeding for *Spea multiplicata* males (blue triangles and dashed line) and females (yellow circles and continuous line). Each point represents an individual spadefoot, and the line and shaded grey area display the linear regression and confidence interval, respectively, for each sex.
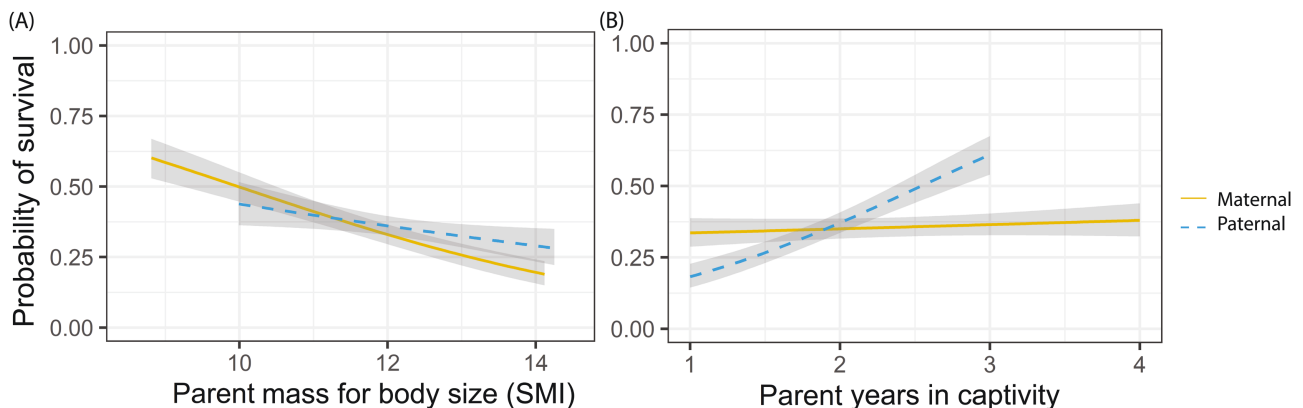
Figure 3. Offspring survival probability vs. parental mass for body size (A) and years in captivity (B) in *Spea multiplicata* from the 2020 experiment. The lines and shaded areas display the logistic regressions with confidence intervals for mothers (yellow continuous line) and fathers (blue dashed line). Abbreviation: SMI, scaled mass index.

($\chi^2_1 = 0.693$, $P = 0.405$; Fig. 3A). For this dataset, there was no evidence of a relationship between maternal years in captivity and offspring survival ($\chi^2_1 = 0.861$, $P = 0.353$), but offspring with fathers that had been in captivity longer were more likely to survive ($\chi^2_1 = 10.139$, $P = 0.00145$; Fig. 3B).

DISCUSSION

Beginning in 2019, after a change in feeding regimen that significantly increased toad mass for body size, we observed low survival of tadpoles from captive breeding attempts of *S. multiplicata*. Throughout > 15 years of working with *S. multiplicata*, in both

naturally and hormonally induced breeding, we typically observed low tadpole mortality in the first few days and, subsequently, high survival of older tadpoles used in experiments. However, in the breeding events described here, we observed massive mortality of captive-bred tadpoles in the first few days, followed by only ~25% survival of tadpoles. Notably, once collections resumed in 2022, we performed a laboratory-based breeding using animals collected from the field only a few weeks earlier. The mass for body size of these adults was consistent with those in the wild, and there was no noticeable mortality in tadpoles. This suggests that parental effects from the temporary new diet regimen for the captive adults, rather than breeding or tadpole husbandry protocols, were responsible for tadpole mortality.

We found that, on average, the adults used in the 2019–2021 captive breeding attempts had substantially higher mass for body size than their wild counterparts, and this high mass for body size was associated with the new feeding regimen (Fig. 1). Our data also indicated that enhanced maternal mass for body size (along with time in captivity) could explain the low survival of tadpoles in captive breeding attempts. Specifically, higher maternal scaled mass index and increasing years in captivity were both associated with decreased offspring survival. In other taxa, maternal diet and obesity can impact the survival of offspring or their risk of obesity (Rivera *et al.*, 2015; Smoothy *et al.*, 2019).

One could argue that we are simply comparing offspring survival of individuals that vary in the extent to which they are obese after overfeeding. As such, we are potentially unable to disentangle the effects of overfeeding (which can alter other aspects of nutrition or the microbiome) vs. excessive mass for body size (obesity). Given that no noteworthy tadpole mortality was observed before the period of overfeeding, we cannot contrast tadpole survival before and during the period of overfeeding to include overfeeding as a factor separate from scaled mass index in our models of tadpole survival. Despite this constraint, our data suggest that excessive maternal mass for body size per se contributes to reduced offspring survival. Our statistical models consistently found that as maternal scaled mass index (a continuous measure) increased, so too did offspring mortality. Presumably, if mass for body size were confounded with other factors associated with overfeeding, we might not have found this predictive relationship. Moreover, offspring survival of mothers with a scaled mass index beyond what was seen in nature (i.e. adults that appeared 'obese') had substantially fewer tadpoles survive in the above experiments (5.4% of 18 964 tadpoles from 51 females) than mothers with a scaled mass index

within the natural range that had been overfed in a similar manner (49.0% of 529 tadpoles from 14 females). Thus, although we cannot state definitively what constitutes an 'obese' spadefoot, nor can we disentangle the effects of overfeeding from obesity per se, our data strongly suggest that excessive maternal mass for body size can reduce offspring survival.

There is less support for an impact of paternal mass for body size on tadpole survival. Our 2019/2021 survival analysis showed that higher paternal scaled mass index was associated with increased offspring survival, which aligns with the potential for higher-condition fathers to produce better-quality offspring (possibly via a 'good genes' effect; Rosenthal, 2017). In our system, *S. multiplicata* males of higher condition are preferred by females and produce more fit offspring (Pfennig, 2000, 2008; Pfennig & Pfennig, 2005; Pfennig & Rice, 2014; Kelly *et al.*, 2019, 2021). However, this relationship was not significant in the 2020 dataset. Furthermore, because paternal mass for body size was positively correlated with time in captivity, we are unable to parse apart the relationship between paternal mass for body size, age (the age of individuals upon collection is unknown) and other effects of captivity on the survival of tadpoles.

Our study highlights the need to consider ecological and evolutionary history when captive-rearing species, especially those, such as spadefoots, that live in 'feast or famine' environments and might be especially prone to developing obesity (Sniderman *et al.*, 2007; Wells, 2007; Genné-Bacon, 2014; Williams & Periasamy, 2020). The way in which animals use nutrition is likely to be impacted by the environmental conditions in which they have evolved (Gilbert & Epel, 2015; West-Eberhard, 2019). Our study species, *S. multiplicata*, has evolved in a highly unpredictable nutritional environment, where adults spend much of the year aestivating underground, emerging aboveground for only a few weeks each year to breed and feed after sporadic summer rains. Spadefoots have therefore evolved a metabolism to conserve energy and store fat (Dimmitt & Ruibal, 1980). When they experience an energy-rich environment, their energy-efficient metabolism is primed to store the abundant food as fat, and in captivity, an overabundance of resources could increase their likelihood of obesity because of a formerly adaptive mechanism. This 'thrifty phenotype hypothesis' (Hales & Barker, 1992, 2001) can thereby explain how phenotypic plasticity in metabolism might place these and similar species (or populations) at particular risk for obesity and obesity-related diseases.

This work also emphasizes that parental effects need not be adaptive. Just as maladaptive mutations can be inherited, effects of maladaptive environments can also be passed down to offspring (reviewed by

Bonduriansky, 2021). The maternal effect observed in our study is a particularly drastic example of a maladaptive parental effect, in which the overfeeding of mothers resulted in offspring being grossly incapable of surviving through larval development.

Ironically, previous studies of these same *S. multiplicata* populations revealed that a condition-dependent maternal effect (in a much reduced form) could be adaptive. In particular, previous studies showed that maternal body condition influences the expression of phenotypic plasticity among their tadpoles. *Spea* tadpoles normally develop into an omnivore morph that feeds mostly on detritus. However, if they are exposed to live prey early in life, they can develop into a distinctive carnivore morph, which specializes on eating fairy shrimp and other tadpoles (Pfennig, 1990, 1992; Levis *et al.*, 2015, 2017a; Harmon *et al.*, 2023). As it turns out, higher-condition females are more likely to produce this novel carnivore phenotype (Pfennig & Martin, 2009; Martin & Pfennig, 2010). Moreover, this maternal effect appears to mediate adaptive divergence between species (specifically, character displacement), in which *S. multiplicata* and *Spea bombifrons* diverge in morph production, hence in resource use (Pfennig & Martin, 2009, 2010). The finding that maternal over-condition is maladaptive is not surprising, given that our captive females had body conditions well beyond what is observed in the wild (Fig. 1). What is unclear, however, is whether over-conditioning creates an entirely new form of maternal effect that decreases tadpole survival or whether over-conditioning drives the pre-existing maternal effect on tadpole morphological development into a pathological state. Clarifying this issue is important because maternal effects are increasingly seen as an important source of phenotypic variation and as being vital to many ecological and evolutionary processes (Moore *et al.*, 2019).

We do not know the underlying mechanisms of the observed parental effects in spadefoot toads. However, our findings that maternal and paternal effects on tadpole survival differ suggest that the two effects are likely to operate by different mechanisms, which deserve further investigation. One possible mechanism by which parental effects are passed to offspring includes epigenetic marks in the germline (e.g. methylation). For instance, transgenerational effects of a maternal high-fat diet in mice appear to be carried by epigenetic marks transferred in the germline, affecting the function of myriad biological processes (e.g. Ng *et al.*, 2010; Dunn & Bale, 2011; Kamimae-Lanning *et al.*, 2015).

Furthermore, it is possible that increased scaled mass index is only one of several side-effects of overfeeding that affect offspring survival deleteriously. Indeed, in our study, individuals that fell within the natural range of scaled mass index still had heightened mortality. Although we performed troubleshooting of tadpole water sources, densities and diet and replaced any equipment that came into contact with tadpoles or their water, these actions did not mitigate tadpole mortality. Thus, other effects of overfeeding (e.g. nutritional or microbial imbalances) could have contributed to the observed tadpole mortality, and further work is necessary to examine this possibility. Nevertheless, we believe that mass for body size serves as a proxy for the ultimate issue of overfeeding, especially given that no other changes were made to protocols.

In terms of practical implications, this work highlights that differences in the natural history, physiology, ecology and evolutionary history of a species (and even the focal population) should be considered when designing appropriate health metrics and care and breeding protocols (Madliger *et al.*, 2016; Pabijan *et al.*, 2020). For instance, the natural mass for body size of *S. multiplicata* differs both within and between populations, depending on local conditions such as the presence of a congener, *S. bombifrons* (Pfennig & Pfennig, 2005). Health and body condition metrics cannot necessarily be shared between sexes or even across populations, let alone species. The protocols and scoring criteria used to identify animals of 'good condition' should be grounded in natural history data at the level of species, population and sex. Well-informed, specific breeding and management schemes are essential for keeping captive animals in appropriate body condition and for the success of captive breeding programmes.

In sum, bigger is not always better when it comes to body condition in frogs and, possibly, in many other animals. Unnatural body conditions could be indicative of other issues attributable to the unnatural conditions of captivity that could accumulate over time. Although there can be a tendency to coddle captive animals, it is essential to understand their natural environments and maintain animals within this realm. We hope that our data will encourage further studies into maladaptive parental effects and, in particular, parental effects of over-conditioning.

DATA AVAILABILITY

The data underlying this article are included in the Supporting Information.

AUTHOR CONTRIBUTIONS

E.A.H. and T.L. are joint first authors. E.A.H. conceived and designed the study, assisted in data analysis and writing the manuscript, and conceived, designed and ran the 2019 and 2021 experiments. T.L. helped to design the study, performed the data analysis and wrote the manuscript. P.W.K. conceived, designed and ran the 2019 and 2020 experiments. C.C. conceived, designed and ran the 2020 experiment. D.W.P. conceived the study and conceived and helped to design the 2019 and 2021 experiments. K.S.P. conceived the study and conceived and helped to design the 2020 and 2021 experiments. All authors contributed to the final version of the paper.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Ananjeva N, **Uteshev V**, **Orlov N**, **Gakhova E. 2015**. Strategies for conservation of endangered amphibian and reptile species. *Biology Bulletin* **42**: 432–439.

Bennett AM, **Murray DL. 2014**. Maternal body condition influences magnitude of anti-predator response in offspring. *Proceedings of the Royal Society B: Biological Sciences* **281**: 20141806. https://doi.org/10.1098/rspb.2014.1806

Bonduriansky R. 2021. Plasticity across generations. In: Pfennig DW, ed. *Phenotypic plasticity and evolution: causes, consequences, controversies*. Boca Raton: CRC Press, 327–348.

Browne RK, **Zippel K. 2007**. Reproduction and larval rearing of amphibians. *ILAR Journal* **48**: 214–234. https://doi.org/10.1093/ilar.48.3.214

Chen C, **Pfennig KS. 2020**. Female toads engaging in adaptive hybridization prefer high-quality heterospecifics as mates. *Science* **367**: 1377–1379. https://doi.org/10.1126/science.aaz5109

Cox N, **Young BE**, **Bowles P**, **Fernandez M**, **Marin J**, **Rapacciuolo G**, **Böhm M**, **Brooks TM**, **Hedges SB**, **Hilton-Taylor C**, **Hoffmann M**, **Jenkins RKB**, **Tognelli MF**, **Alexander GJ**, **Allison A**, **Ananjeva NB**, **Auliya M**, **Avila LJ**, **Chapple DG**, **Cisneros-Heredia DF**, **Cogger HG**, **Colli GR**, **de Silva A**, **Eisemberg CC**, **Els J**, **Fong GA**, **Grant TD**, **Hitchmough RA**, **Iskandar DT**, **Kidera N**, **Martins M**, **Meiri S**, **Mitchell NJ**, **Molur S**, **Nogueira CC**, **Ortiz JC**, **Penner J**, **Rhodin AGJ**, **Rivas GA**, **Rödel M-O**, **Roll U**, **Sanders KL**, **Santos-Barrera G**, **Shea GM**, **Spawls S**, **Stuart BL**, **Tolley KA**, **Trape J-F**, **Vidal MA**, **Wagner P**, **Wallace BP**, **Xie Y. 2022**. A global

reptile assessment highlights shared conservation needs of tetrapods. *Nature* **605**: 285–290.

Densmore CL, **Green DE. 2007**. Diseases of amphibians. *ILAR Journal* **48**: 235–254. https://doi.org/10.1093/ilar.48.3.235

Dimmitt MA, **Ruibal R. 1980**. Exploitation of food resources by spadefoot toads (*Scaphiopus*). *Copeia* **1980**: 854–862. https://doi.org/10.2307/1444465

Dunn GA, **Bale TL. 2011**. Maternal high-fat diet effects on third-generation female body size via the paternal lineage. *Endocrinology* **152**: 2228–2236. https://doi.org/10.1210/en.2010-1461

Ferrie GM, **Alford VC**, **Atkinson J**, **Baitchman E**, **Barber D**, **Blaner WS**, **Crawshaw G**, **Daneault A**, **Dierenfeld E**, **Finke M**, **Fleming G**, **Gagliardo R**, **Hoffman EA**, **Karasov W**, **Klasing K**, **Koutsos E**, **Lankton J**, **Lavin SR**, **Lentini A**, **Livingston S**, **Lock B**, **Mason T**, **McComb A**, **Morris C**, **Pessier AP**, **Olea-Popelka F**, **Probst T**, **Rodriguez C**, **Schad K**, **Semmen K**, **Sincage J**, **Stamper MA**, **Steinmetz J**, **Sullivan K**, **Terrell S**, **Wertan N**, **Wheaton CJ**, **Wilson B**, **Valdes EV. 2014**. Nutrition and health in amphibian husbandry. *Zoo Biology* **33**: 485–501. https://doi.org/10.1002/zoo.21180

Finke MD. 2015. Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology* **34**: 554–564. https://doi.org/10.1002/zoo.21246

Gascon C, **Collins JP**, **Moore RD**, **Church DR**, **McKay JE**, **Mendelson JR III (eds)**. **2007**. *Amphibian conservation action plan*. IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, UK. 64pp.

Genné-Bacon EA. 2014. Thinking evolutionarily about obesity. *The Yale Journal of Biology and Medicine* **87**: 99–112.

Gilbert SF, **Epel D. 2015**. *Ecological developmental biology: integrating epigenetics, medicine, and evolution*. Sunderland: Sinauer.

Hales CN, **Barker DJ. 1992**. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* **35**: 595–601. https://doi.org/10.1007/BF00400248

Hales CN, **Barker DJ. 2001**. The thrifty phenotype hypothesis. *British Medical Bulletin* **60**: 5–20. https://doi.org/10.1093/bmb/60.1.5

Harmon EA, **Evans B**, **Pfennig DW. 2023**. Frog hatchlings use early environmental cues to produce an anticipatory resource-use phenotype. *Biology Letters* **19**: 20220613. https://doi.org/10.1098/rsbl.2022.0613

Jayson S, **Harding L**, **Michaels CJ**, **Tapley B**, **Hedley J**, **Goetz M**, **Barbon A**, **Garcia G**, **Lopez J**, **Flach E. 2018**. Development of a body condition score for the mountain chicken frog (*Leptodactylus fallax*). *Zoo Biology* **37**: 196–205. https://doi.org/10.1002/zoo.21409

Kamimae-Lanning AN, **Krasnow SM**, **Goloviznina NA**, **Zhu X**, **Roth-Carter QR**, **Levasseur PR**, **Jeng S**, **McWeeney SK**, **Kurre P**, **Marks DL. 2015**. Maternal high-fat diet and obesity compromise fetal hematopoiesis. *Molecular Metabolism* **4**: 25–38. https://doi.org/10.1016/j.molmet.2014.11.001

Kaplan RH. 1987. Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*. *Oecologia* **71**: 273–279. https://doi.org/10.1007/BF00377295

Kaplan RH. 1998. Maternal effects, developmental plasticity, and life history evolution. An amphibian model. In: Mousseau T, Fox CW, eds. *Maternal effects as adaptations*. New York: Oxford University Press, 244–260.

Kaplan RH, **King EG. 1997**. Egg size is a developmentally plastic trait: evidence from long term studies in the frog *Bombina orientalis*. *Herpetologica* **53**: 149–165.

Kaplan RH, **Salthe SN. 1979**. The allometry of reproduction: an empirical view in salamanders. *The American Naturalist* **113**: 671–689. https://doi.org/10.1086/283425

Keller CB, **Shilton CM. 2002**. The amphibian eye. *Veterinary Clinics: Exotic Animal Practice* **5**: 261–274.

Kelly PW, **Pfennig DW**, **de la Serna Buzón S**, **Pfennig KS. 2019**. Male sexual signal predicts phenotypic plasticity in offspring: implications for the evolution of plasticity and local adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **374**: 20180179.

Kelly PW, **Pfennig DW**, **Pfennig KS. 2021**. A condition-dependent male sexual signal predicts adaptive predator-induced plasticity in offspring. *Behavioral Ecology and Sociobiology* **75**: 1–8.

Kouba A, **Vance CK. 2009**. Applied reproductive technologies and genetic resource banking for amphibian conservation. *Reproduction, Fertility and Development* **21**: 719–737.

Ledon-Rettig CC, **Pfennig DW**, **Nascone-Yoder N. 2008**. Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies. *Evolution & Development* **10**: 316–325.

Levis NA, **de la Serna Buzon S**, **Pfennig DW. 2015**. An inducible offense: carnivore morph tadpoles induced by tadpole carnivory. *Ecology and Evolution* **5**: 1405–1411. https://doi.org/10.1002/ece3.1448

Levis NA, **Martin RA**, **O'Donnell KA**, **Pfennig DW. 2017a**. Intraspecific adaptive radiation: competition, ecological opportunity, and phenotypic diversification within species. *Evolution; International Journal of Organic Evolution* **71**: 2496–2509. https://doi.org/10.1111/evo.13313

Levis NA, **Serrato-Capuchina A**, **Pfennig DW. 2017b**. Genetic accommodation in the wild: evolution of gene expression plasticity during character displacement. *Journal of Evolutionary Biology* **30**: 1712–1723. https://doi.org/10.1111/jeb.13133

MacCracken JG, **Stebbings JL. 2012**. Test of a body condition index with amphibians. *Journal of Herpetology* **46**: 346–350. https://doi.org/10.1670/10-292

Madliger CL, **Cooke SJ**, **Crespi EJ**, **Funk JL**, **Hultine KR**, **Hunt KE**, **Rohr JR**, **Sinclair BJ**, **Suski CD**, **Willis CK**, **Love OP. 2016**. Success stories and emerging themes in conservation physiology. *Conservation Physiology* **4**: cov057. https://doi.org/10.1093/conphys/cov057

Martin RA, **Pfennig DW. 2010**. Maternal investment influences expression of resource polymorphism in amphibians: implications for the evolution of novel resource-use phenotypes. *PLoS One* **5**: e9117. https://doi.org/10.1371/journal.pone.0009117

McWilliams DA. 2008. *Nutrition recommendations for some captive amphibian species (Anura and Caudata)*. The Canadian Association of Zoos and Aquariums Nutrition Advisory and Research Group. http://www.amphibianark.org/wp-content/uploads/2018/07/Amphibian-nutrition-report-CAZA-2008.pdf.

Moore MP, **Whiteman HH**, **Martin RA. 2019**. A mother's legacy: the strength of maternal effects in animal populations. *Ecology Letters* **22**: 1620–1628. https://doi.org/10.1111/ele.13351

Ng S-F, **Lin RC**, **Laybutt DR**, **Barres R**, **Owens JA**, **Morris MJ. 2010**. Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* **467**: 963–966. https://doi.org/10.1038/nature09491

Okamiya H, **Hayase N**, **Kusano T. 2021**. Increasing body size and fecundity in a salamander over four decades, possibly due to global warming. *Biological Journal of the Linnean Society* **132**: 634–642. https://doi.org/10.1093/biolinnean/blaa201

Pabijan M, **Palomar G**, **Antunes B**, **Antoł W**, **Zieliński P**, **Babik W. 2020**. Evolutionary principles guiding amphibian conservation. *Evolutionary Applications* **13**: 857–878. https://doi.org/10.1111/eva.12940

Passos LF, **Garcia G**, **Young RJ. 2018**. Comparing the bacterial communities of wild and captive golden mantella frogs: implications for amphibian conservation. *PLoS One* **13**: e0205652. https://doi.org/10.1371/journal.pone.0205652

Peig J, **Green AJ. 2009**. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* **118**: 1883–1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x

Peig J, **Green AJ. 2010**. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Functional Ecology* **24**: 1323–1332. https://doi.org/10.1111/j.1365-2435.2010.01751.x

Pessier AP. 2014. Short tongue syndrome and hypovitaminosis A. In: Mader D, Divers S, eds. *Current therapy in reptile medicine and surgery*. Philadelphia: Saunders, 271–276.

Pfennig DW. 1990. The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* **85**: 101–107. https://doi.org/10.1007/bf00317349

Pfennig DW. 1992. Proximate and functional causes of polyphenism in an anuran tadpole. *Functional Ecology* **6**: 167–174. https://doi.org/10.2307/2389751

Pfennig DW, **Martin RA. 2009**. A maternal effect mediates rapid population divergence and character displacement in spadefoot toads. *Evolution; International Journal of Organic Evolution* **63**: 898–909. https://doi.org/10.1111/j.1558-5646.2008.00544.x

Pfennig DW, **Martin RA. 2010**. Evolution of character displacement in spadefoot toads: different proximate mechanisms in different species. *Evolution; International Journal of Organic Evolution* **64**: 2331–2341. https://doi.org/10.1111/j.1558-5646.2010.01005.x

Pfennig DW, **Rice AM. 2007**. An experimental test of character displacement's role in promoting postmating isolation between conspecific populations in contrasting competitive environments. *Evolution; International Journal of Organic Evolution* **61**: 2433–2443. https://doi.org/10.1111/j.1558-5646.2007.00190.x

Pfennig KS. 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. *Behavioral Ecology* **11**: 220–227. https://doi.org/10.1093/beheco/11.2.220

Pfennig KS. 2008. Population differences in condition-dependent sexual selection may promote divergence in non-sexual traits. *Evolutionary Ecology Research* **10**: 763–773.

Pfennig KS, **Chunco AJ**, **Lackey AC. 2007**. Ecological selection and hybrid fitness: hybrids succeed on parental resources. *Evolutionary Ecology Research* **9**: 341–354.

Pfennig KS, **Pfennig DW. 2005**. Character displacement as the 'best of a bad situation': fitness trade-offs resulting from selection to minimize resource and mate competition. *Evolution; International Journal of Organic Evolution* **59**: 2200–2208.

Pfennig KS, **Rice AM. 2014**. Reinforcement generates reproductive isolation between neighbouring conspecific populations of spadefoot toads. *Proceedings of the Royal Society B: Biological Sciences* **281**: 20140949. https://doi.org/10.1098/rspb.2014.0949

Rahbek C. 1993. Captive breeding—a useful tool in the preservation of biodiversity? *Biodiversity & Conservation* **2**: 426–437.

R Core Team. **2018**. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Rivera HM, **Kievit P**, **Kirigiti MA**, **Bauman LA**, **Baquero K**, **Blundell P**, **Dean TA**, **Valleau JC**, **Takahashi DL**, **Frazee T**, **Douville L**, **Majer J**, **Smith MS**, **Grove KL**, **Sullivan EL. 2015**. Maternal high-fat diet and obesity impact palatable food intake and dopamine signaling in nonhuman primate offspring. *Obesity (Silver Spring, Md.)* **23**: 2157–2164. https://doi.org/10.1002/oby.21306

Rosenthal GG. 2017. *Mate choice*. Princeton: Princeton University Press.

Salthe SN, **Duellman WE. 1973**. Quantitative constraints associated with reproductive mode in anurans. In: Vial JL, ed. *Evolutionary biology of the anurans*. 229–249. Columbia: University of Missouri Press.

Sandoz MA, **Wages M**, **Wooten KJ**, **Clendening SL**, **Smith LR**, **Mulhearn B**, **Smith PN. 2017**. Surface water mitigates the anti-metamorphic effects of elevated perchlorate concentrations in New Mexico spadefoot toad larvae (*Spea multiplicata*). *Environmental Science and Pollution Research* **24**: 17839–17844. https://doi.org/10.1007/s11356-017-9436-0

Seidl F, **Levis NA**, **Schell R**, **Pfennig DW**, **Pfennig KS**, **Ehrenreich IM. 2019**. Genome of *Spea multiplicata*, a rapidly developing, phenotypically plastic, and desert-adapted spadefoot toad. *G3: Genes, Genomes and Genetics* **9**: 3909–3919.

Shilton CM, **Smith DA**, **Crawshaw GJ**, **Valdes E**, **Keller CB**, **Maguire GF**, **Connelly PW**, **Atkinson J. 2001**. Corneal lipid deposition in Cuban tree frogs (*Osteopilus septentrionalis*) and its relationship to serum lipids: an experimental study. *Journal of Zoo and Wildlife Medicine* **32**: 305–319.

Silla AJ, **Calatayud NE**, **Trudeau VL. 2021**. Amphibian reproductive technologies: approaches and welfare considerations. *Conservation Physiology* **9**: coab011. https://doi.org/10.1093/conphys/coab011

Smoothy J, **Larcombe AN**, **Chivers EK**, **Matthews VB**, **Gorman S. 2019**. Maternal high fat diet compromises survival and modulates lung development of offspring, and impairs lung function of dams (female mice). *Respiratory Research* **20**: 21.

Sniderman AD, **Bhopal R**, **Prabhakaran D**, **Sarrafzadegan N**, **Tchernof A. 2007**. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. *International Journal of Epidemiology* **36**: 220–225. https://doi.org/10.1093/ije/dyl245

Steiger S. 2013. Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. *Proceedings of the Royal Society B: Biological Sciences* **280**: 20131225.

Welch AM, **Semlitsch RD**, **Gerhardt HC. 1998**. Call duration as an indicator of genetic quality in male gray tree frogs. *Science* **280**: 1928–1930. https://doi.org/10.1126/science.280.5371.1928

Wells JCK. 2007. Commentary: why are South Asians susceptible to central obesity? The El Niño hypothesis. *International Journal of Epidemiology* **36**: 226–227. https://doi.org/10.1093/ije/dyl287

West-Eberhard MJ. 2019. Nutrition, the visceral immune system, and the evolutionary origins of pathogenic obesity. *Proceedings of the National Academy of Sciences of the United States of America* **116**: 723–731.

Williams R, **Periasamy M. 2020**. Genetic and environmental factors contributing to visceral adiposity in Asian populations. *Endocrinology and Metabolism (Seoul, Korea)* **35**: 681–695. https://doi.org/10.3803/EnM.2020.772

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article on the publisher's website.

Table S1: contains the body measurements of parents used in this paper.
Table S2: contains the survival data for tadpoles used in this paper.
Table S3: contains measurements of spadefoot toads from the field.
Table S4: includes measurements of females in the colony before the period of overfeeding.