



## Original Article

# Parental care is beneficial for offspring, costly for mothers, and limited by family size in an egg-feeding frog

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Care that parents provide to offspring often comes at the cost of other reproductive opportunities, generating parent–offspring conflict and sibling rivalry whenever there are resource shortfalls. Although these family dynamics are most often studied in birds, convergent family structures can be found in diverse lineages, including in frogs that feed their developing young with unfertilized trophic eggs. We used observations in a captive colony of the strawberry poison frog (*Oophaga pumilio*) to test the hypothesis that parental care is a limiting resource within families of this egg-feeding frog, and thus could generate conflicts of interest. We found that maternal provisioning was valuable to offspring: Trophic egg provisioning was positively associated with larval survival, size at metamorphosis, and postmetamorphic survival. Maternal care came at the cost of other reproductive opportunities: Females produced fewer reproductive clutches when caring for tadpoles than when dependent young were absent. Even under presumably benign captive conditions, resources were limited within reproductive cycles. Tadpoles in larger broods received smaller meals, and although survival increased across brood sizes of 1–3 tadpoles, the largest broods (4 tadpoles) suffered reduced survival. Finally, older tadpoles received larger meals, a pattern consistent with the prediction that females would favor their most valuable offspring. These results suggest that relationships within *O. pumilio* families are constrained by resource limitation. Because of a convergent family structure coupled with substantial metabolic and natural history differences, egg-feeding frogs will provide an excellent complement to birds when addressing the causes and consequences of conflicts of interest within families.

**Key words:** brood size, Dendrobatidae, egg feeding, parental favoritism, parent–offspring conflict, trophic egg.

## INTRODUCTION

Parents across diverse lineages improve the fitness prospects of their offspring via postzygotic care, often in the form of exogenous food deliveries that fuel the development of a helpless neonate, nestling, or larvae into a juvenile that can more or less fend for itself (Royle et al. 2012). The extent of care parents provide is an important feature of life history, as allocating resources to an offspring often comes at the cost of other reproductive opportunities (Williams 1966; Trivers 1972). The costly nature of parental investment, along with relatedness asymmetries within families, will typically create conflicts of interest over the level and division of care (Trivers 1974; Mock and Parker 1997). Within reproductive cycles, these conflicts are exacerbated by incentives for parents to initially produce more offspring than they are able or willing to rear

to independence (Mock and Forbes 1995). The many links among life history, social relationships, and ecology have made the family an arena in which theoreticians and empiricists alike have generated and tested hypotheses about how complex behavioral and life-history traits evolve (Mock and Parker 1997; Wright and Leonard 2002; Royle et al. 2012).

The hypothesis that resource limitation drives proximate and evolutionary conflicts among family members has chiefly been tested in birds (Mock and Parker 1997; Wright and Leonard 2002; Royle et al. 2012). This taxonomic focus is understandable, as parental feeding of offspring is common in birds, and food deliveries are often frequent and relatively straightforward to observe and assign to individual offspring. The importance of food in shaping families, however, will not necessarily be comparable across taxonomic groups (Trumbo 2012). For example, the intense and immediate metabolic demands of growing nestlings are thought to limit avian brood size in a number of ways (Skutch 1949; Lack 1954). However, the nutritional requirements of growing endotherms

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vastly outpace those of larval invertebrates, fishes, and amphibians (Newman 1992; Brust 1993; Burd et al. 2006), and thus, nutrient availability should limit family size and generate sibling competition to a lesser extent in these ectotherms. Nonavian examples that prove the rule have provided compelling examples of similar constraints shaping dissimilar families (e.g., Mock 1985; Burd et al. 2006) and have also offered novel insights into the selective pressures driving the evolution of parental care (Kutschera and Wirtz 2001; Brown et al. 2010; Trumbo 2012).

A feature of avian reproduction that has proven particularly useful for asking questions about how within-family relationships evolve is the (at least partial) control that parent birds have over how resources are allocated among their concurrent offspring (Wright and Leonard 2002; Mock et al. 2011). This degree of parental control is relatively rare in animals (Smiseth and Moore 2002; Mas et al. 2009). Potentially compelling parallel examples come from frogs, where the provisioning of offspring with unfertilized eggs has evolved independently in several lineages (Crump 1996; Perry and Roitberg 2006; Summers et al. 2006). In the strawberry poison frog (*Oophaga pumilio*), a mother deposits 1–4 tadpoles individually in tiny, nutrient-poor, water bodies (usually leaf axils), a behavior that presumably gives mothers complete control over within-brood allocation of the trophic eggs she delivers regularly to her developing tadpoles (Brust 1990, 1993; Maple 2002). When a mother visits, her tadpole vibrates rapidly, a display perhaps analogous to the begging of young birds and other animals, and one presumed to have evolved in the context of offspring competition for limited resources (Weygoldt 1980; Stynoski 2012). What remains unknown about this or any similar frog, however, is the extent to which nutrients are actually a limited commodity within families. Because *O. pumilio* mothers feed on superabundant leaf litter invertebrates (Donnelly 1991), can buffer any stochastic fluctuations in prey availability by storing resources in the form of trophic eggs, and rear starvation-resistant offspring (tadpoles can survive weeks without food: Brust 1993; Killius and Dugas 2014), the assumption of food-limitation merits skepticism.

We used observations in a captive colony of *O. pumilio* to test the hypothesis that parental care is a limiting resource within families of this egg-feeding frog. Testing for such limitation is important not only for understanding the extent to which nuclear families across taxonomic groups are shaped by similar constraints but also because such limitation has been assumed to drive both sexual and parent–offspring conflicts in this and similar systems (Brown 2013). We began by examining the relationship between parental provisioning and offspring success, a relationship that is assumed across taxonomic groups, but much less often tested (Schwagmeyer and Mock 2008). Tadpoles are by all accounts entirely dependent on trophic eggs during development, but whether trophic eggs beyond the minimum required for metamorphosis are valuable to offspring remains unresolved (Brust 1990, 1993; Maple 2002). We built on previous work by considering the relationships between maternal feeding and larval survival, juvenile size at metamorphosis (a good fitness proxy: Altwegg and Reyer 2003), and postmetamorphic survival. Two pieces of evidence suggest that care comes at the cost of current and future reproduction for females, and thus potentially limiting: Breeding frequency is negatively associated with long-term survival in captivity (Dugas et al. 2015), and field observations suggest that females cease mating while caring for young (Pröhl and Hödl 1999; Haase and Pröhl 2002). We re-examined this second cost by comparing the production of reproductive clutches when females were caring for tadpoles to clutch production when

dependent young were absent, taking advantage of the opportunity to monitor this parameter more completely than would be practical in the field. Whether resource limitation operates within reproductive cycles is a question not previously addressed. We looked for evidence of such limitation by comparing meal size and tadpole metamorphic success across broods of different sizes. Finally, we tested for parental favoritism, predicted when demand for care exceeds supply (Mock and Parker 1997; Mock et al. 2011). More developed tadpoles are likely more valuable, especially when predation on tadpoles is high (Maple 2002) because more developed tadpoles have fewer remaining days of exposure and may be protected by accumulated, maternally provisioned alkaloids (Stynoski, Torres-Mendoza, et al. 2014; Stynoski, Shelton, et al. 2014). These observations will provide a clearer picture of whether resource limitation structures proximate and ultimate relationships within *O. pumilio* families.

## METHODS

### Study species

*Oophaga pumilio* is a small terrestrial frog native to the Caribbean slope of Central America, from Nicaragua to Panama. In the wild, males defend territories from which they court females, and both sexes mate with multiple partners (Pröhl and Hödl 1999). Following successful courtship, the female lays a reproductive clutch of ~5 eggs in the leaf litter. Reproductive clutches are composed of fertilized (i.e., reproductive) eggs and are tended by a male until hatching. The female then transports her new tadpoles to rearing sites, typically water-filled leaf axils (Weygoldt 1980; Brust 1993; Pröhl and Hödl 1999; Pröhl 2005). She visits tadpoles regularly throughout development, supplying unfertilized trophic eggs on which tadpoles seem to be entirely dependent for nutrition (Weygoldt 1980; Brust 1993; Maple 2002).

In the wild and in captivity, tadpoles are typically found alone in rearing sites (i.e., without nurserymates). Rarely, two or more tadpoles are found together. Little is known about how or why more than one tadpole is deposited in the same rearing site, but in all observed cases (including those in our colony), only one tadpole ever completes development (Brust 1990; Dugas MB, unpublished data). Thus, for our purposes, we considered these as single events (i.e., scored them as only one tadpole) for questions of survival and ignored them for questions of trophic egg feeding (these comprised 6 of 300 tadpole observations). We operationally defined a brood as all tadpoles that parents transported to rearing sites within 7 days, which corresponds to two consecutive censuses (see below).

### Captive breeding conditions and monitoring reproduction

We established a captive breeding colony using wild-caught individuals from 4 populations in the Bocas del Toro Region of Panama: Tranquilo Bay, Isla Bastimentos: 9°15'8.03"N, 82°8'43.30"W; Cemetery, Isla Bastimentos: 9°20'48.48"N, 82°12'23.04"W; Isla Popa: 9°8'25.98"N, 82°7'39.11"W; Aguacate Peninsula: 9°12'47.13"N, 82°12'49.29"W. Although breeding pairs considered here included within-population, between-population, and F1 backcross pairs, pair make-up does not influence reproductive rate or success (Dugas and Richards-Zawacki 2015). We housed breeding pairs (one male, one female) in 37 × 22 cm (base) × 25 cm (height) plastic enclosures maintained in an environmental chamber at 22–27 °C and ~40% relative humidity under a 12L/12D light

cycle (similar to conditions in Bocas del Toro). Each tank was lined with sheet moss and filled with live plants (*Philodendron* spp.). Water-filled PVC tubes (10 height  $\times$  3.8 cm diameter,  $\sim$ 20 mL) in each corner of the tank served as tadpole deposition and rearing sites; 4 is the maximum brood size reported in the wild (Brust 1990). These artificial rearing sites provided the only standing water in tanks and thus were the only potential sites for tadpole deposition. We misted tanks twice daily and fed pairs with  $\sim$ 50 adult *Drosophila melanogaster* and/or *Drosophila hydei* 3 times weekly. Mistlers were on automatic timers, and flies were provided through small openings in the lid, so both tasks could be accomplished without disturbing adults (further details provided in Dugas et al. 2013). When a tadpole completed metamorphosis, we moved it to a smaller rearing tank, where we supplemented the fruit fly diet with smaller springtails (Collembola).

Throughout the study period, we used regular censuses (detailed below) to confirm that all adults and juveniles were alive and to inspect each rearing site for the presence of tadpoles or newly metamorphosed juveniles. Adult *O. pumilio* do not move tadpoles after initial deposition (Maple 2002; Stynoski 2009), and so individuals were readily identifiable (as tadpoles and new juveniles) by their rearing site. We defined a tadpole as “dead” when we found remains or when it was missing on two consecutive censuses, in which case we considered the tadpole to have died on the first date it was missing. We found remains for about half the tadpoles that died. The intensity of our monitoring efforts and the number of pairs used varied during the study period. For clarity, we describe below the monitoring methods and statistical analyses we used to answer each of our questions grouped by question, rather than chronology.

### Benefits of trophic eggs to tadpoles

To test the prediction that maternal trophic egg provisioning would be positively associated with tadpole fitness proxies, we monitored trophic egg provisioning by 28 mother frogs between 13 March and 30 June, 2014. Observations in the wild indicate that feedings occur about every two days, typically in the morning (Brust 1990; Maple 2002; Pröhl 2005; Stynoski 2012), and so we inspected every rearing site three times weekly at 1200 (the midpoint of the 12-h light portion of the light:dark cycle). To quantify trophic egg provisioning, we emptied the contents of each rearing site into a shallow plastic dish without removing the tadpole. Staging and measuring tadpoles would have been ideal, but pilot work revealed that such handling produced high mortality. For each rearing site, we counted 1) trophic eggs and 2) jelly capsules. Except very late in development, tadpoles consume only egg yolk, and so empty jelly capsules can be used to detect eggs that tadpoles consumed between checks (Maple 2002). We returned trophic eggs (and water) to the rearing tube and discarded jelly capsules, allowing us to estimate meal size at each check as: meal size = (eggs + jelly capsules) – (eggs present on previous check), and total provisioning to a tadpole as the sum of all meals. With this conservative estimate of maternal provisioning and tadpole egg consumption, we assume 1) that all trophic eggs are leftover from previous events unless they are explicitly accounted for as jelly capsules and 2) that we detected evidence of all eggs provisioned/consumed (i.e., that tadpoles consumed no jelly capsules and none decayed). Because jelly capsules might be consumed and might decay, total counts are likely to underestimate total trophic egg consumption, although not in a systematic way. If and when tadpoles completed metamorphosis ( $N = 30$ ), we weighed them to the nearest 0.01 g on an electronic balance. Nine of these 30 juveniles were immediately euthanized as part of an

unrelated study, and we transferred all others to individual juvenile rearing tanks.

We began by asking whether the number of trophic eggs a tadpole received predicted survival to the juvenile stage. We converted provisioning to a per day rate because tadpoles that died early would presumably be fed fewer total eggs. For this analysis, we used a generalized linear mixed model (GLMM) with binary error structure, included the fixed effect of eggs/day, and the random effects of parent pair and brood nested within pair (no adult was included in more than one pair in this sample). Degrees-of-freedom for fixed effects in this and all analyses described below were calculated with the Kenward–Roger approximation (Kenward and Roger 1997). For the 30 tadpoles that completed metamorphosis, we then used a linear mixed model (LMM) to assess the relationships between total trophic eggs provisioned and juvenile mass or days to metamorphosis, again including random effects as described above. For the 21 juveniles that were not sacrificed, we asked whether trophic egg provisioning was associated with the probability of surviving 90 days post-metamorphosis using a GLMM with a binary error distribution. We defined 90 days as the end of our observation period because after this period siblings were placed together to address space constraints. Three families contributed multiple observations; for all three families, juveniles came from the same brood and had the same survival fate, so we used means for each family in the final analysis (final  $N = 16$ ). Death date was not recorded for one juvenile that died before 90 days, but for the other 7 individuals that died (from 6 parent pairs), we examined the relationship between trophic egg provisioning and days survived with a correlation; we again took the mean for the family with two observations.

### Costs of offspring feeding to mothers: reproduction during periods of parental care

To test the prediction that caring for tadpoles would come at the cost of decreased reproduction, we monitored the reproductive clutch production of 45 *O. pumilio* pairs with twice weekly censuses from 4 October 2012 to 20 May 2013. To minimize disturbance to adults, we restricted egg searching to  $\sim$ 2 min, which was enough time to examine most potential clutch deposition sites, but was likely not exhaustive. When we detected a clutch, we noted its size (number of eggs) and individually marked it by placing a small colored wire tag (pipe cleaner) near the clutch (e.g., on a leaf stem).

To test the prediction that parental care comes at the cost of other reproductive opportunities, we compared females' reproductive clutch and reproductive egg production (converted to per day rates) when tadpoles were present to their production when tadpoles were absent, using females from the subset of pairs that produced  $\geq 1$  tadpole during the study period ( $N = 32$ ). Because previous work in the field suggested that mating would cease entirely when tadpoles were present (Pröhl and Hödl 1999; Haase and Pröhl 2002), we were conservative in assigning clutches to the “tadpoles present” condition; if we found clutches on the same census date we found new tadpoles or new metamorphs, we assumed these clutches were not laid while tadpoles were present. We tested the prediction that reproduction would slow when mothers were caring for tadpoles using a repeated-measures LMM with reproductive clutch or egg production per day entered as the dependent variable and tadpoles present (yes/no) as a fixed effect. We included the random effects of male and female population-of-origin. We then compared the size of first broods (ones that appeared when no other tadpoles were present) to the size of second broods that overlapped with them; we



did not include in this analysis any broods that may have then overlapped with the second brood (i.e., second broods were not later considered first broods). To compare first and second brood sizes, we used a GLMM with a Poisson error distribution. We entered brood size as the dependent variable, brood rank (first or second) as a fixed factor and included the G-side random effects of male and female population and the R-side random effect of pair.

### Density dependence of parental care: effects of brood size

To test the prediction that per capita parental care would be negatively associated with offspring number (i.e., parental care is limiting within a reproductive cycle), we examined the relationships between brood size (a brood is the group of siblings that appear in rearing sites within 7 days) and metrics of tadpole care and success. From 20 July 2012 to 6 December 2013, we tracked the fate of every tadpole produced in the colony. We used this sample to examine the relationships between brood size and two measures of brood outcome: 1) number of juveniles produced and 2) within-brood survivorship, defined as the number of juveniles produced per number of tadpoles. We tested these using GLMMs, specifying a negative binomial error distribution for juvenile number and a binomial error distribution for juveniles/tadpoles. In both models, we included the fixed effects of brood size and the quadratic term “brood size  $\times$  brood size” to accommodate the possibility that the marginal costs of additional brood members accrue in a nonlinear fashion.

We examined the relationship between brood size and the duration of the larval period using LMMs. We entered mean larval duration of juveniles from the brood (days from deposition to metamorphosis) as the dependent variable, and tested the fixed effect of either 1) tadpole number (brood size) or 2) the number of juveniles eventually resulting from the brood (number of offspring parents successfully raised). Although we tested the quadratic for both fixed effects, neither was significant or improved model fit, and so we do not present these in Results. We included the random effects of male and female population and parent pair (in this sample, no individual was involved in more than one pair). We excluded several broods for which the accuracy of deposition and metamorphosis dates were unclear (e.g., when tadpoles were first detected at an advanced developmental stage or metamorphs were found outside their rearing tubes).

Broods sometimes overlapped, and we developed and explored an additional factor distinguishing broods that did not overlap with another from those that were the older or younger of overlapping broods. However, for clarity, we do not present results including this factor because it was never significant in analyses described above (all  $P > 0.840$ ), and would be difficult to interpret because it did not account for the duration of overlap (which may have differed for individual tadpoles) or continuous overlap among multiple broods.

Finally, we used observations of trophic egg provisioning (described above) to test the prediction that tadpoles in larger families would receive smaller meals. We used a GLMM with a Poisson error distribution (in all cases, we compared model fit of Poisson and negative binomial, and in this case, the latter model did not converge). Instead of “brood size,” we used the finer-grained fixed effect of the number of tadpoles present on the day of the meal as well as the fixed effect of tadpole age on the day of the meal. By including tadpole age in the model, we were able to test for a nonrandom association between this likely proxy for offspring reproductive value and the extent of maternal provisioning. To account for nonindependence of observations from the same family and of the same

tadpole, we entered the random effects of parent pair and tadpole nested within parent pair. To minimize the chances of mistakenly counting eggs from more than one parental feeding event as a single meal, we restricted this analysis to meals that were 1) estimated with observations no more than 48 h apart (i.e., roughly 2/3 of the observations, we made with thrice weekly checks) or 2) estimated without counting jelly capsules (i.e., no eggs were present on one check and only eggs were present on the next). We used SAS v9.2 (SAS Institute, Cary, NC) for all analyses. All tests are 2-tailed, and means are reported  $\pm$  standard deviation unless otherwise noted.

## RESULTS

### Benefits of trophic eggs to tadpoles

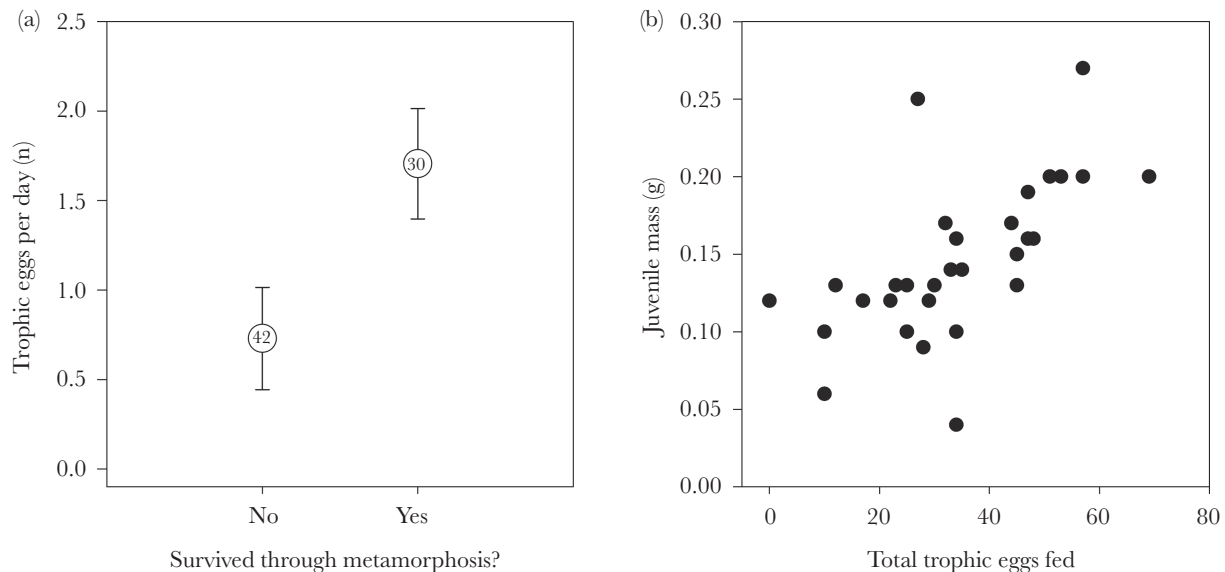
We followed trophic egg provisioning to 72 tadpoles and detected provisioning (trophic eggs or jelly capsules) to 54 of these. When we did not detect provisioning to a tadpole, this presumably indicates that the tadpole was abandoned entirely or fed little and infrequently. Tadpoles for which we detected provisioning were significantly more likely to complete metamorphosis than tadpoles for which we did not (fed: 29/54, not fed: 1/18; Yates  $\chi^2_1 = 5.21$ ,  $P = 0.023$ ). Overall, tadpoles were fed  $1.14 \pm 1.00$  eggs/day. The number of trophic eggs tadpoles received was positively associated with the probability that a tadpole would complete metamorphosis ( $F_{1,70} = 13.20$ ,  $P = 0.005$ ; Figure 1), a pattern that was similar but marginal if we removed from the analysis any tadpoles for which we never detected provisioning ( $F_{1,53} = 3.83$ ,  $P = 0.058$ ). In the tadpoles that did complete metamorphosis, total trophic egg count was positively associated with juvenile mass ( $F_{1,25.1} = 11.86$ ,  $P = 0.002$ ; Figure 1). However, trophic egg count was not associated with time until metamorphosis ( $F_{1,28} = 0.96$ ,  $P = 0.336$ ). Juveniles that survived  $\geq 90$  days were fed more eggs than those that did not (survived:  $37.2 \pm 11.4$  eggs, died:  $18.5 \pm 9.8$  eggs;  $t$ -test:  $t_{14} = -3.35$ ,  $P = 0.005$ ), a difference that was similarly significant in a general linear model (likelihood ratio  $\chi^2_1 = 10.55$ ,  $P = 0.001$ ). Trophic eggs provisioned and days survived were positively but marginally correlated in tadpoles that died within 90 days ( $r = 0.787$ ,  $N = 6$ ,  $P = 0.063$ ).

### Costs of offspring feeding to mothers: reproduction during periods of tadpole care

Pairs that produced tadpoles ( $N = 32$ ) spent  $45 \pm 24\%$  of the study period with tadpoles present. Twenty-one of these females produced reproductive clutches that we detected while caring for tadpoles. When tadpoles were present in rearing sites, both the number of reproductive clutches ( $F_{1,56.1} = 17.52$ ,  $P < 0.001$ ) and reproductive eggs produced ( $F_{1,55.4} = 14.97$ ,  $P < 0.001$ ) were lower (Figure 2). These results were similar when we restricted analysis to pairs that produced at least one clutch under both conditions (clutches/day:  $F_{1,29.1} = 10.46$ ,  $P = 0.003$ ; eggs/day:  $F_{1,31.9} = 9.15$ ,  $P = 0.005$ ). In 19 of these families (25 events), new tadpoles appeared when older tadpoles were already present; 34% of all broods overlapped with another, with  $26 \pm 13$  (range: 11–61) days between the oldest tadpoles of the two broods. First broods were  $0.5 \pm 1.4$  tadpoles larger than second broods although this difference was statistically marginal ( $F_{1,48} = 3.75$ ,  $P = 0.059$ ).

### Density dependence of parental care

We observed 167 broods reared by 43 females, with a mean brood size of  $1.9 \pm 1.0$  tadpoles (46% of broods contained a single tadpole, 26% contained 2 tadpoles, 20% contained 3 tadpoles, and



**Figure 1**

The number of trophic eggs that *Oophaga pumilio* mothers provided to tadpoles that did and did not survive to complete metamorphosis, converted to a per day rate (a), and the relationship between the total number of eggs provisioned to a tadpole throughout development and its mass at metamorphosis, presented necessarily only for those individuals that reached this milestone (b). Error bars in (a) indicate 95% confidence intervals and sample sizes appear inside points indicating means.

8% contained 4 tadpoles). The number of brood members that completed metamorphosis was associated with both the linear ( $F_{1,164} = 15.07$ ,  $P = 0.001$ ) and quadratic ( $F_{1,164} = 8.78$ ,  $P = 0.004$ ) effects of brood size. Similarly, juveniles/tadpoles was significantly associated with both the linear ( $F_{1,161} = 8.05$ ,  $P = 0.005$ ) and quadratic ( $F_{1,161} = 8.85$ ,  $P = 0.003$ ) effects of brood size. Larger broods produced more juveniles and a higher proportion of juveniles in large broods completed metamorphosis, but both effects were true only up to a brood size of 3, when the direction of this relationship reversed (Figure 3). We examined the average larval duration of juveniles from 85 broods reared by 28 pairs. Larval duration was positively associated with brood size although this effect was statistically marginal ( $F_{1,81.2} = 3.72$ ,  $P = 0.057$ ; Figure 3). The relationship between juvenile number and larval duration was again positive but was nonsignificant ( $F_{1,79.6} = 2.02$ ,  $P = 0.159$ ).

We examined the relationship between meal size, family size, and tadpole age with 113 observed meals to 47 tadpoles from 25 families. Meal size was negatively associated with the number of offspring being raised by females ( $\beta \pm \text{standard error [SE]} = -0.182 \pm 0.056$ ;  $F_{1,49.1} = 10.44$ ,  $P = 0.002$ ) and was positively associated with tadpole age ( $\beta \pm \text{SE} = 0.010 \pm 0.004$ ;  $F_{1,110} = 4.88$ ,  $P = 0.029$ ; Figure 4).

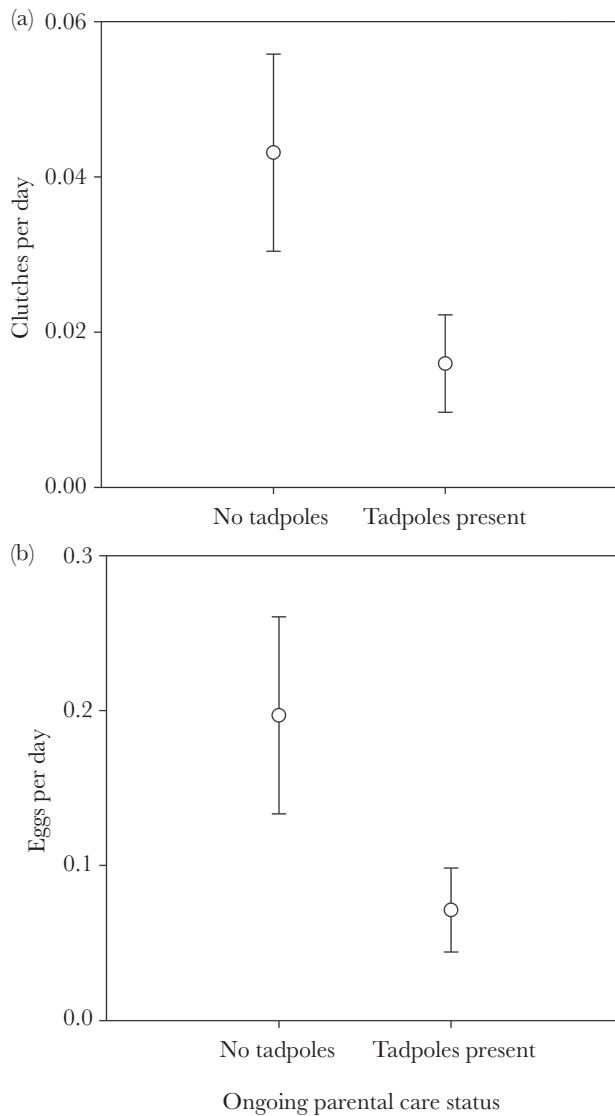
## DISCUSSION

Our observations of *O. pumilio* reproduction in captivity suggest within-family resource limitation and opportunities for conflicts of interest, paralleling the superficially similar avian families more familiar in studies of parental care. We have confirmed that maternal trophic eggs are beneficial for offspring, positively influencing larval survival, size at metamorphosis, and postmetamorphic survival. We demonstrated that caring for tadpoles comes at the cost of other current reproductive opportunities for females, complementing the earlier finding that frequent reproduction is associated with reduced survival in this frog (Dugas et al. 2015). While the relationship between parental care and brood size was complex,

we found several lines of evidence consistent with within-brood resource limitation, including smaller meal size in larger broods and reduced tadpole survival in the largest broods. Because food is available ad libitum and rearing sites can be visited quickly and at minimal energetic cost, it seems most likely that captive females are less resource limited than wild ones. However, it remains possible that other features of the captive environment, including reduced territory size, forced monogamy, and a restricted diet (but see Dugas et al. 2013), limit females in unknown ways. Of paramount importance in the future will be detailed observational and experimental work in the wild, where it will be possible to explore the extent to which such benefits of, costs of, and limitations on parental investment explain variation in fitness under natural selective regimes.

Previous work has suggested that maternally provisioned eggs influence *O. pumilio* tadpole fitness only in a discontinuous manner, either allowing tadpoles to complete metamorphosis or not (Maple 2002). Instead, our results indicate that the benefits of care to tadpoles accrue in a continuous fashion, with maternal provisioning above the minimum required for metamorphosis improving both the pre- and postmetamorphic fitness prospects of offspring, even under captive conditions. Although heritable components of quality likely also contribute to the association between maternal provisioning and offspring success (Dugas and Richards-Zawacki 2016), carryover effects of the developmental environment are common in animals (Kasumovic 2013). Data demonstrating a relationship between fitness across life stages are rare for amphibians (Cabrera-Guzmán et al. 2013; Tarvin et al. 2015), but such relationships are key determinants of the fitness payoffs that shape the strategies of both parents and offspring (Trivers 1972; Moore et al. 2015). In the wild, the strength of the relationship between maternal provisioning and offspring fitness will depend on the relative importance of various sources of mortality (e.g., starvation or predation), and these remain uninvestigated.

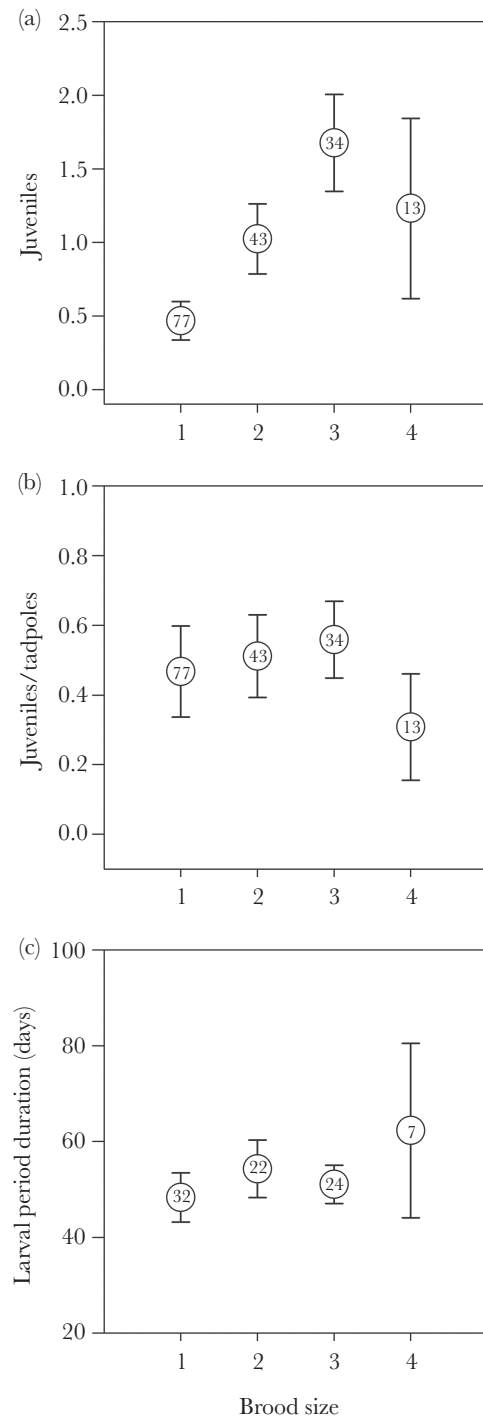
Although relatively high levels of maternal provisioning positively influence survival and size in young *O. pumilio*, they do not appear



**Figure 2**  
Reproductive clutches (a) and reproductive eggs (b) laid by 32 *Oophaga pumilio* mothers while tadpoles were absent in rearing sites and while dependent tadpoles were present. Error bars indicate 95% confidence intervals.

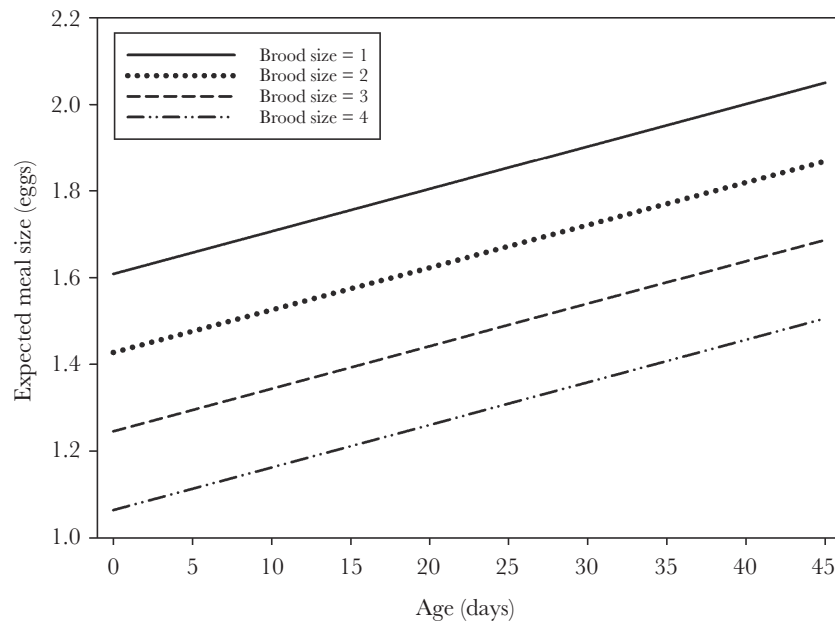
to speed larval development (Brust 1993; Maple 2002; this paper). In one population, trophic egg counts were even negatively associated with developmental speed (Maple 2002), surprising given that high predation pressure on tadpoles (as high as 68% depredated: Maple 2002) should favor rapid development (Wilbur and Collins 1973). The substantial plasticity common in larval amphibian development (Newman 1992; Morey and Reznick 2000; Warkentin 2011) might set the stage for weaning conflict in egg-feeding frogs, with offspring preferring to devote resources to growth and mothers preferring that nutrients be directed toward reaching independence (Trivers 1974; Mock and Parker 1997). Similar trade-offs operate in frogs that care for clutches, with protected larvae enjoying the benefits of leisurely development within the egg, but attending to the risks of predation or desiccation and speeding the transition to independence when appropriate (Warkentin 2011; Delia et al. 2013).

Mothers made fewer reproductive attempts while caring for tadpoles, indicating that the care *O. pumilio* mothers provide comes at



**Figure 3**  
Relationships between brood size and the number of juveniles produced by a brood (a), the proportion of the brood that completed metamorphosis (b), and the average duration of the larval period for brood members that completed metamorphosis (c). Error bars indicate 95% confidence intervals and sample sizes appear inside points indicating means.

the cost of other reproductive opportunities. However, mating did not cease entirely, as has been reported in the field (Pröhl and Hödl 1999; Haase and Pröhl 2002). This difference between field and laboratory observations seems most likely to reflect relaxed nutritional constraints in captivity and/or lower detection of mating



**Figure 4**

Expected meal size for tadpoles over mean developmental period (45 days) when mothers are simultaneously caring for 1, 2, 3, or 4 tadpoles. Expected values, presented to illustrate the direction and size of effects, were derived from a GLMM that included the fixed effects of brood size and tadpoles age and the random effects of mother and tadpole nested within mother.

events in the field. High levels of male attention (or harassment) in the relatively confined quarters of captivity might also result in females mating more often than they would in the wild. Proximately, this cost of parental care might be mediated by absolute limits on egg production or by physiological trade-offs (Dugas et al. 2015). These constraints could also be less direct: Females might slow or stop reproductive clutch production because even if these clutches were successful, females might not be able to effectively care for any additional young.

Meals were smaller in larger broods, and larval duration was marginally longer. But larger broods were more successful up to broods of 3, a pattern consistent with higher-quality females creating larger broods (Verhulst et al. 1995). At the largest brood size of 4 (the maximum observed in the wild: Brust 1990), success declined, suggesting that the maximum output of maternal care is limited even when females are not calorie limited and visits to tadpoles require little to no meaningful energetic costs. When resources are limited, parental favoritism is expected (Mock and Parker 1997), and consistent with this prediction, we found that mothers provided larger meals to older tadpoles (contra Brust 1990). Intriguingly, the behavioral displays performed by larvae of taxonomically diverse egg-feeding frogs (Jungfer 1996; Kam and Yang 2002) are performed more intensely by older tadpoles in *O. pumilio*, and future work can address the role of this behavior in mediating nonrandom maternal allocation (Stynoski 2012).

In both our captive colony and in the wild (Maple 2002), mothers often abandoned tadpoles, either ceasing feeding abruptly or never feeding some individuals. In captivity, we could exclude the possibility of female mortality, and so these observations strongly suggest that the production and subsequent abandonment of offspring is a regular feature of *O. pumilio* reproduction. This phenomenon is not uncommon in animals, and apparently, superfluous offspring can serve an insurance function against early losses, for example, to predators (Mock and Forbes 1995; Forbes and Mock 2000). Such insurance offspring could be valuable to *O. pumilio* mothers because their

presence would minimize time females spent not reproducing (Pröhl and Hödl 1999; Haase and Pröhl 2002). Furthermore, the marginal maintenance costs of insurance tadpoles should be low, as tadpoles can survive weeks without feeding (Brust 1990, 1993; Killius and Dugas 2014). This hypothesis could be tested both in the wild and in captivity by asking whether feeding to abandoned tadpoles resumes after other offspring are removed (Forbes and Mock 2000).

Despite metabolic and ecological differences between frogs and more frequently studied birds and mammals, resource limitation seems as good a candidate to limit family size and drive within-family conflicts of interest in egg-feeding frogs as it is in endothermic vertebrates (Mock and Parker 1997). Before egg-feeding frogs can become a model system for studying parent-offspring conflict and communication, careful behavioral observations and modern molecular techniques must be employed to describe family structure in the wild. For example, if tadpole predation is high (Maple 2002), it may be the case that mothers rarely, if ever, rear large broods to independence, reducing the extent to which within-brood limitation actually drives conflict.

The diversity of parental care behaviors in frogs (Crump 1996; Summers et al. 2006) offers comparative opportunities to address the causes and consequences of postzygotic parental investment, as do populations of the same species occupying different habitats (e.g., Pröhl 2005). Such exciting directions await further study of basic natural history, and detailed quantification of the costs and benefits of care will help strengthen and inform comparative efforts.

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