Tadpole begging behavior and parent-offspring interactions in the mimic poison frog

by

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Studies on offspring begging behavior have focused on whether the behavior communicates offspring need and guides parental food provisioning in the context of honest signaling theory. However, despite the plethora of empirical work on this issue applied to avian systems, begging occurs in the absence of direct sibling interactions in some species, and so may not be selected for solely in the context of intrabrood dynamics, as traditionally hypothesized. Here we broaden the taxonomic scope of investigations into the phenomenon by studying begging behavior in the Peruvian mimic poison frog (*Ranitomeya imitator*). This system presents several advantages, including individually housed and cared for offspring (eliminating the confounding influence of sibling competition on begging behavior). We specifically investigated in *R. imitator*, a) whether begging is an honest signal of need, or a signal of quality (or neither), b) if begging is costly, and c) if food-provisioning by parents depends on offspring need. First, under manipulation of long-term diet, cumulative nutritional deficits significantly affected begging behavior, with food-limited tadpoles begging increasingly more over the course of development. In a second experiment, tadpoles induced to beg suffered a cost of taking longer to reach developmental marker stages. Compared to the control group, begging tadpoles also had marginally lower growth rates. Finally, in an experiment testing parental response to tadpoles experiencing different levels of nutrition, parents were more likely to feed the tadpole that did not receive any supplemental food over its food-supplemented sibling. Congruently, the non-supplemented tadpoles were on average fed relatively more frequently. Together, the findings from this study suggest that in the mimic poison frog, offspring begging behavior acts as an honest signal of offspring need and influences how provisioning parents differentially allocate food among their offspring.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: Laboratory experiments testing offspring signaling theory

INTRODUCTION

In altricial animals where parents must invest energy in providing resources to postnatal offspring, selection will favor the distribution of these resources to offspring in a manner that maximizes the reproductive returns from parental investment. Therefore, parents should adjust care efforts in response to signals that express reliable information on the expected offspring-specific returns for those efforts (Godfray 1995). In the context of honest signaling theory (Zahavi 1975), begging has been hypothesized to be one such reliable signal. Provisioning has evolved as the principal form of parental care in birds and mammals, but rarely in other vertebrates (Rosenblatt 2003). Hence, investigations of begging behavior have largely focused on these taxa (avian: Budden and Wright 2001, Sacchi et al. 2002, Glassey et al. 2014; mammalian: Brotherton et al. 2001, Smiseth and Lorentsen 2001). These studies usually aim to test three conditions initially proposed by Godfray (1991, 1995) as those that would allow begging to persist as an evolutionarily stable strategy: 1) begging intensity reflects offspring need, 2) parents provide resources in relation to offspring begging intensity, and 3) begging is costly. However, many previous studies aiming to test these predictions may not have fully isolated the function of begging behavior and its importance in parent-offspring communication.

First, functions of offspring begging (particularly those of interest to the caregiving parent[s]) may be obscured by the effects of interactions between offspring within broods (Rodríguez-Gironés et al. 1996). Although offspring begging displays may have evolved in response to broodmate competition over the allocation of parental care, including food provisioning (Mock and Parker 1998, Budden and Wright 2001), evidence that begging in some species occurs in the absence of direct broodmate interactions suggests that the behavior is not driven primarily by intrabrood competition, as some have argued (Rodríguez-Gironés et al. 1996). In birds and mammals, parents typically care for multiple offspring in a single brooding site (e.g. a nest; but see Smiseth and Lorentsen 2001, Quillfeldt 2002). Thus, differential levels of begging in these taxa may reflect sibling competition (Rodríguez-Gironés et al. 1996) and/or relatedness (Briskie et al. 1994), in addition to offspring-specific information. Therefore, species like the mimic poison frog (*Ranitomeya imitator*) that care for individual offspring in separate rearing sites allow the communicative role of offspring begging to be isolated in the specific context of parent-offspring interactions. More accurate characterization of the information signaled by an offspring individual to its parent(s) may allow for more reliable inquiries into whether (and how) it is used by parents during care delivery (e.g. Quillfeldt 2002; Smiseth and Lorentsen 2001).

Secondly, not all aspects of offspring condition that can potentially be signaled by begging behavior have been considered. Several studies have investigated begging behavior as a means of signaling information regarding offspring hunger to provisioning parents. However, most of these studies have observed or manipulated short-term offspring hunger alone, assuming that only this aspect of the individual's condition is reflected in begging intensity (Mas and Kölliker 2011; Royle et al. 2002). Fewer studies have investigated less ephemeral aspects of offspring condition (Weary and Fraser 1995, Saino et al. 2000), like long-term hunger and energy availability measured over the entire course of offspring development, as potential determinants of begging intensity (see Price et al. 1996).

Finally, offspring begging has been tested predominantly as a signal of need, following the traditional assumption that begging intensity is higher in weaker, more "needy" offspring (as traditionally hypothesized; Godfray 1991, 1995). As Mock et al. (2011) point out as an overlooked yet plausible alternative, variation in begging intensity may instead be caused by variation in individual offspring quality, allowing individuals with higher metabolic energy reserves to sustain higher levels begging (i.e. “signal of quality”; Grafen 1990). Higher quality offspring likely reach larger body sizes and thereby higher survival and fitness, which then ultimately can contribute to reproductive success on the parents’ behalf. It is crucial to distinguish between these alternative hypotheses concerning the information signaled through begging, because if the distribution of parental care depends on offspring begging levels, the proximate basis of variation in begging level will suggest the ultimate basis of selection on parental investment in particular offspring.

Considering that parental care is rare among amphibians, only occurring in 10 - 20% of extant species (Lehtinen et al. 2003), most of which are found in tropical latitudes (Magnusson and Hero 1991), parent-offspring interactions have only recently been explored in these taxa. This study extends research on offspring signaling and parental care behavior beyond the scope of avian and mammalian taxa to a tropical amphibian, specifically the mimic poison frog, *Ranitomeya imitator*, found in northeastern Peru (Caldwell and Summers 2003). Expanding the taxonomic breadth of comparative analyses is instructive in discovering whether key factors underlie the evolution of begging signals across the tree of life, or whether begging evolves in an idiosyncratic manner with different factors driving the evolution of signals in different taxa.

Increasing attention has been paid to mother-offspring communication in the context of trophic egg-provisioning in several tropical anuran species (*Colostenthus beebei*: Bourne et al. 2001; *Dendrobates vanzolinii*: Caldwell and Oliveira 1999; *Anotheca spinosa*: Jungfer and Weygoldt 1999; *Chirixalus eiffingeri*: Kam and Yang 2002; *Oophaga pumilio*: Stynoski and Noble 2012; Weygoldt 1980). However, compared to these species, *R. imitator* differs in several key, reproductive life history traits: 1) social and genetic monogamy, 2) biparental care, 3) tadpoles individually occupy separate pools (shared with *O. pumilio*), and 4) trophic (unfertilized) egg-provisioning is orchestrated by males (Brown et al. 2010).

Study objectives and hypotheses

Using this "single-offspring, single-site" system of biparental care, this study focused on offspring begging behavior in *R. imitator* in the general context of honest signaling theory. Because begging behavior was observed strictly in terms of begging time, the term "begging effort" (rather than "begging intensity") is hereafter applied to the theoretical framework described above. Specifically, the study tested the following hypotheses modified from Godfray's offspring signaling theory (1991, 1995):

1) *Begging effort reflects offspring energy reserves*:
    a) Begging is a signal of offspring need, where begging duration is longer in smaller or less developed (lower life stage) offspring, or
    b) Begging is a signal of offspring quality, where begging duration is longer in larger or more developed (higher life stage) offspring, or
    c) Begging is a signal of neither need nor quality.
2) *Begging incurs a cost to offspring energy reserves or development.*
3) *Parental food-provisioning depends on offspring energy reserves:*
    a) The parent provides greater care to needier offspring (smaller and/or less developed offspring), or
    b) The parent provides greater care to higher-quality offspring (larger and/or more developed offspring), or
    c) The parent provides equal care to offspring regardless of need or quality.

METHODS

Study species

Ranitomeya imitator is a long lived, diurnal poison frog found in montane wet forest (Caldwell and Summers 2003) and primary lowland tropical moist forest of north-central Peru (Icochea et al. 2004). The species breeds year-round with occasional pauses under abnormally warm or dry conditions or heavy rainfall. The male calls to bring the female to a breeding site, and following egg-fertilization, the female deposits her clutch on stems or leaves, sometimes above phytotelmata (small pools that form in stem or leaf axils). The male carries each tadpole (12 – 14 d post egg-hatch) of the clutch individually on its back to separate development sites (usually phytotelmata). While the male continues to attend the clutch over the entire course of tadpole development (2 – 3 mos), he periodically (roughly every 7 d) calls to the female to initiate trophic (unfertilized) egg provisioning. Upon arrival of a parent, the tadpole displays a vibration behavior similar to that observed in other anuran species (*Colostenthus beebei*: Bourne et al. 2001; *Oophaga pumilio*: Stynoski and Noble, 2012); it ceases normal swimming movements and vibrates its body vigorously in one place. The male stimulates egg-provisioning by calling repeatedly, and each provisioning episode can last over 3.5 h (see Chapter 2).

In order to test the hypotheses described above, three separate experiments were conducted:

Experiment 1: Begging effort reflects offspring energy reserves.

To determine whether tadpole begging effort reflects offspring body size and/or developmental life stage in *R. imitator* and whether the relationship between these variables is consistent with the hypothesis that begging is a signal of offspring need, or alternatively that it is a signal of offspring quality, tadpole begging time was monitored while nutrient availability was manipulated in otherwise identical artificial pools. Tadpoles were bred from an ongoing captive nursery of *R. imitator* adults indigenous to north-central Peru. Five similar-aged, adult mating pairs housed in separate terraria each produced three experimental tadpoles from separate clutches ($n = 5$).

At 14d post-hatch, the age at which tadpoles roughly begin to display begging behavior (M. Yoshioka, personal observation), each experimental tadpole was placed into a polypropylene plastic container filled with 25 mL of filtered, distilled water and subjected randomly to one of three food treatments (each tadpole produced by an adult pair assumed one of the three treatments): a constant mass of 0.005g administered at different frequencies of one, four, and six times a week, equating to 0.005, 0.02, and 0.03g a week. The tadpole food consisted of a mixture of fatty-acid-enriched fish flakes (Cyclopeeze [Argent Chemical Laboratories, Redmond, WA]; OmegaOne First Flakes [Omega, LLC, Sitka, AK]), which are readily accepted by laboratory tadpoles as an alternative to maternally-derived trophic eggs. Laboratory temperature was held constant at 25ºC, and accumulation of excrement and debris was minimized via water changes performed once every 7 d at hour 1700 for all tadpole pools, and adult parents were kept absent from the experiment.

Begging behavior of each experimental tadpole was observed at hours 0900 – 1100 using the following procedure: To initiate the onset of tadpole begging, a random, unrelated adult female was placed into the tadpole for a total of 7 min. To prevent the female from escaping, a transparent piece of Plexi-glass was placed directly on top of the container. The first minute was used to allow the tadpole to acclimate to the presence of the adult, after which the tadpole’s behavior was recorded by a high-definition video camera positioned directly above the glasscovered container. The experimenter stood away from the camera during recording so as not to disturb tadpole behavior.

Following video recording, the adult female was removed from the container and artificial tadpole food in the amount corresponding to food treatment was added to the pool. Video recordings of tadpole behavior were later reviewed remotely using the ethogram-based program JWatcher (Blumstein et al. 2006). During these video reviews, a tadpole was considered to be begging when it vibrated its whole body vigorously in one place within the water column. Normal swimming patterns were ignored. For each tadpole, the total time it spent begging during the 6 min was recorded for each of the five days of observation. This parameter of total begging time per begging episode is hereafter referred to as the metric for “begging effort”. Using the above procedure, begging effort of each tadpole was recorded weekly, coinciding with the days on which all tadpoles were fed, marking tadpole ages 18, 25, 32, 39, and 46d post-hatch.

To confirm the effect of food treatment on tadpole body size, dorsal surface area (cm^2) of each tadpole (as described in Davis et al. 2008) was measured every day begging effort was observed. Dorsal surface area (hereafter referred to as “DSA”) was used to monitor tadpole body size because repeatedly weighing tadpoles can be injurious and harmful to the animals. To accurately weigh tadpoles, they must be periodically removed from water, and the resulting desiccation for as little as 2 min can be stressful for a tadpole (Davis et al. 2008). The depth between the dorsum and ventrum of *R. imitator* tadpoles does not significantly change over the course of development; therefore, body depth was predicted not to confound estimates of body size. On the other hand, since tadpole developmental stage potentially influences body size, the life stage (derived from Gosner stages; Gosner 1960) of each tadpole was also recorded.

Experiment 1 statistical analyses.

Whether food treatment had an effect on tadpole body size and life stage was analyzed using a generalized least squares (GLS) linear mixed effects models (“GLSLMM”) with DSA (and life stage separately) as the response and food treatment as the factor. To account for the temporal autocorrelation of DSA for each tadpole, a within-tadpole autoregressive (AR1) correlation structure was applied to tadpole identity. The same GLSLMM method with the within-tadpole AR1 correlation structure was employed to test for the separate effects of food treatment and tadpole body size on begging time.

Experiment 2: Begging incurs a cost to offspring energy reserves.

To test the assumption that offspring begging imposes a cost on offspring energy reserves, tadpole begging effort was manipulated to isolate the effect of begging on tadpole growth and development. Tadpoles were subjected randomly to one of two treatments, “no begging” and “begging”, while being fed a constant diet throughout development. Tadpoles were bred from the captive nursery described above. Five similar-aged, adult mating pairs housed in separate terraria each contributed two tadpoles from independent clutches (*n* = 5). At 14 d post-hatch, each tadpole was placed into a polypropylene plastic container filled with 25 mL of filtered, distilled water. While a constant diet of 0.012g per week of artificial food (see Experiment 1 methods) was maintained across treatments, one tadpole (experimental) of a sibling pair was manipulated to beg to an adult female four times a week, while the other tadpole (control) was introduced to the “no begging” treatment in which it was never exposed to an adult female and thus not induced to beg.

Each experimental tadpole was manipulated to beg through contact with an adult female (see Experiment 1 methods) at 0900 – 1100 h four times a week, marking 20 tadpole ages throughout development. The 6-min video recordings of tadpole behavior were reviewed using the program JWatcher, and begging effort was recorded for each begging episode. Begging effort was recorded in this experiment to test if begging time during the 6-min monitoring period remained similar across all experimental tadpoles, given that begging time is impossible to precisely control. Although control tadpoles were never induced to beg, the procedure of placing the Plexi-glass cover on top of the tadpole container, positioning the container under the video camera, and video-recording behavior for 6 min was administered to control tadpoles four times a week. Videos were reviewed using JWatcher to confirm that these tadpoles did not elicit any signs of begging.

To monitor tadpole energy reserves, body size and life stage (derived from Gosner stages; Gosner 1960) of each tadpole was recorded on each of the 20 observation days throughout tadpole development. Dorsal surface area (DSA) was once again used to estimate tadpole body size. Laboratory temperature was kept at 25ºC and water changes of tadpole pools were performed once every 7 d at hour 1700 to prevent accumulation of excrement and debris. Adult parents were kept absent from the experiment.

Experiment 2 statistical analyses.

The effect of treatment (“begging” vs. “no begging”) on tadpole body size was analyzed using a GLS linear mixed effects model (“GLSLMM”) with DSA as the response and treatment as a factor. To account for the temporal autocorrelation of DSA for each tadpole, an autoregressive (AR1) correlation structure was applied to tadpole identity. The same GLSLMM method with the within-tadpole AR1 correlation structure was used to test for a treatment effect on tadpole life stage.

Experiment 3: Parental food-provisioning depends on offspring energy reserves.

To determine whether parental investment provided to a developing tadpole depends on offspring energy reserves, egg-provisioning naturally carried out by parental adults was monitored under manipulation of tadpole nutrient availability. Tadpoles were bred from the captive nursery described above. Eleven similar-aged, adult mating pairs housed in separate terraria each cared for two tadpoles belonging to the same egg clutch (i.e. two sibling tadpoles) (n = 11). Each adult was fed 15 - 20 fruit flies coated in a vitamin and calcium supplement (Repashy Calcium Plus [Repashy Ventures, Inc., La Jolla, CA]) and each terrarium held at 25ºC and misted with filtered, distilled water daily.

Within a family, both sibling tadpoles had access to the trophic eggs provided by the adult female; however, one sibling was chosen randomly to also have access to supplementary food: 0.006g of artificial tadpole food (see Experiment 1 methods) administered five times a week for a total of 0.03g per wk. Tadpoles subjected to this diet collectively represented the "supplemented" group, while their siblings that were not food-supplemented represented the "non-supplemented" group.

Males individually deposited each sibling tadpole into a 250-mL, straight-sided, clear glass jar, and although each terrarium contained only two jars (to discourage production of more than two sibling tadpoles), any extra tadpoles that were subsequently deposited were immediately removed from the terrarium. Accumulation of excrement and debris in tadpole jars was minimized via water changes performed daily at hour 1700.

For each sibling tadpole starting at 4d post-hatch, tadpole body size (dorsal surface area [DSA]) and life stage were recorded daily. To quantify egg-provisioning, presence or absence of trophic eggs in the tadpole pool was recorded daily as a binary variable: 0 for not fed and 1 for fed.

The ventral skin of *R. imitator* tadpoles is largely transparent, enabling observations of the egg digestion process. During initial stages of digestion, the egg can be observed in the right side of the intestine (from a perpendicular view of the tadpole dorsum). The egg then eventually moves to the left side before being completely digested, at which point it is no longer visible within the gut coils (L. Schulte, personal communication, M. Yoshioka, personal observation). Therefore, gut coil observations confirmed the presence of egg-feeding in the event that the tadpole consumed the egg before the day's observations began (from hour 0900 – 1000 every day).

Experiment 3 statistical analyses.

The effect of food treatment ("supplemented" vs. "non-supplemented") on tadpole body size was analyzed using a GLS linear mixed effects model ("GLSLMM") with DSA as the response and treatment as a factor. To account for the autocorrelation of DSA over time, an autoregressive (AR1) correlation structure was applied to tadpole identity. The same GLSLMM method with the AR1 correlation structure was used to test for a food treatment effect on tadpole life stage.

The effect of food treatment on the binary response of egg-feeding (0: not fed or 1: fed) was analyzed using a generalized mixed effects model with a binomial error distribution and logit link function. This model tested separately the effect of food treatment and tadpole life stage on the probability of feeding by incorporating them as independent fixed factors. In analyzing the effect of tadpole life stage on egg-feeding, a linear regression (egg-feeding as a function of life stage) was first fit by a generalized linear mixed effects model ("$\text{GLMM}_{\text{prob}}$"

hereafter refers to the GLMM predicting probability of feeding). However, a quadratic regression (egg-feeding as a function of life stage + life stage^2) improved model parsimony and thus, a generalized quadratic mixed effects model ("GQMM$_{prob}$" hereafter refers to the GQMM predicting probability of feeding) was ultimately employed.

The effect of food treatment on feeding frequency (frequency of the binary egg-feeding response receiving a value of 1: fed by tadpole life stage) was analyzed using a generalized mixed effects model with a Poisson error distribution and log link function. This model incorporated food treatment and tadpole life stage as fixed factors. To test the effect of tadpole life stage on feeding frequency, a linear regression was first fit by a generalized linear mixed effects model ("GLMM$_{freq}$" hereafter refers to the GLMM predicting feeding frequency). However, a quadratic regression (feeding frequency as a function of life stage + [life stage]^2) improved model-fit and thus a generalized quadratic mixed effects model ("GQMM$_{freq}$ hereafter refers to the GQMM predicting feeding frequency) was ultimately employed.

In all GQMMs, both tadpole DSA (mean DSA values calculated for each tadpole life stage for GQMM$_{freq}$) and life stage were initially included as covariates of food treatment. Tadpole identity and parental pair identity were included as random factors (tadpole "ID" and "parent"). For ease of comparing between factor levels, coefficient estimates of significant factors were converted from log-odds estimated by the GQMM$_{prob}$ and log-counts estimated by the GQMM$_{freq}$ to probability and count values, respectively.

All statistical analyses were performed using the program R v. 2.15.1 (R Development Core Team). Model selection employed a reverse stepwise-regression approach and the Akaike Information Criterion (AIC) (which also validated correlation structures when applicable). Likelihood ratio tests were used to rank model parsimony. In analyzing all coefficient estimates, level of statistical significance was set *a priori* at $p < 0.05$. All laboratory protocols were approved by East Carolina University's Institutional Animal Care and Use Committee (AUP permit #D295).

RESULTS

Experiment 1

Tadpoles in all three food treatments had significantly positive growth rates (dorsal surface area [“DSA”] as a function of age) (GLSLMM: low food: *ß* = 0.0095, *SE* = 0.0011, $p <$ 0.0001, medium: *ß* = 0.0243, *SE* = 0.0012, $p < 0.0001$, high: *ß* = 0.0280, *SE* = 0.0050, $p =$ 0.0002). Since tadpole life stage was positively correlated with age (Pearson’s product-moment correlation: low food: $t_{23} = 14.0327$, $p < 0.0001$, medium food: $t_{23} = 7.9436$, $p < 0.0001$, high food: $t_{23} = 6.3336$, $p < 0.0001$), life stage as a covariate of DSA was left out of the GLSLMM testing for the effect of food treatment on DSA. Tadpole growth rate under the low food treatment was significantly lower than that under the other two treatments (GLSLMM: tadpole age × $\text{treatment}_{\text{med}}$: $t_{69} = 3.8600$, $p = 0.0003$, tadpole age × $\text{treatment}_{\text{high}}$: $t_{69} = 4.8893$, $p < 0.0001$ [low food = reference level]). The medium and high food treatments did not differ in tadpole growth rates (GLSLMM: tadpole age × treatment: $t_{69} = 1.0295$, $p = 0.3069$ [medium food = reference level]).

Tadpoles in all three food treatments had significantly positive development rates (life stage as a function of age) (GLSLMM: low food: *ß* = 0.1429, *SE* = 0.0140, $p < 0.0001$, medium: *ß* = 0.0243, *SE* = 0.0041, $p < 0.0001$, high: *ß* = 0.0023, *SE* = 0.0058, $p < 0.0001$). From the beginning of the experiment (18d post-hatch; Kruskal-Wallis Chi-squared test: $X^2 = 10.8571$, $p =$ 0.0044) tadpoles in the low food treatment were significantly less advanced in life stage (Supplementary Table 1) compared those in the higher food treatments. However, tadpole development in the low food treatment significantly accelerated compared to rates resulting from the higher food treatments (Supplementary Table 1). The medium and high food treatments did not differ in tadpole development rate (GLSLMM: age × treatment: $t_{69} = -1.4231$, $p = 0.1592$ [medium food = reference level]).

While tadpole begging time was similar across all food treatments at the beginning of the experiment (Pearson's Chi-squared test: $X^2 = 0.7015$, $p = 0.9511$), begging time in the low food treatment significantly increased with tadpole age (Figure 1, Table 1), while begging time in the other two treatments remained relatively constant throughout tadpole development (GLSLMM: medium food: $ß = -0.0022$, $SE = 0.0032$, $p = 0.4999$, high: $ß = 0.0010$, $SE = 0.3977$, $p = 0.6773$). Begging time did not differ between the medium and high food treatments throughout the entire experiment (GLSLMM: age × treatment: $t_{69} = 0.3806$, $p = 0.7047$).

Post-hoc analyses revealed that the positive effect of tadpole DSA on begging time was significantly higher in the low food treatment compared to those resulting from the other two treatments (GLSLMM: DSA × $\text{treatment}_{\text{med}}$: $t_{69} = -3.3625$, $p = 0.0013$, DSA × $\text{treatment}_{\text{high}}$: $t_{69} = -3.233$, $p = 0.0019$ [low food = reference level]). The medium and high food treatments did not differ in the effect of tadpole DSA on begging time (GLSLMM: DSA × $\text{treatment}_{\text{high}}$: $t_{69} = 0.4844$, $p = 0.6297$ [medium food = reference level]). Similar trends were found for the effect of tadpole life stage on begging time (GLSLMMs: low food as reference: life stage × $\text{treatment}_{\text{med}}$: $t_{69} = -2.2989$, $p = 0.0245$, life stage × $\text{treatment}_{\text{high}}$: $t_{69} = -1.7914$, $p = 0.0776$; medium food as reference: DSA × $\text{treatment}_{\text{high}}$: $t_{69} = 0.4233$, $p = 0.6734$).

Experiment 2

Tadpole life stage was positively correlated with age (Pearson's product-moment correlation: begging: $t_{138} = 30.6591$, $p < 0.001$, no begging: $t_{138} = 23.4664$, $p < 0.0001$); therefore, it was excluded as a covariate from the final GLSLMM used to test for a treatment effect on tadpole DSA. In both treatments, tadpoles had significantly positive growth rates (DSA as a function of age) (GLSLMM: begging: *ß* = 0.0138, *SE* = 0.0014, $p < 0.0001$, no begging: *ß* = 0.0147, *SE* = 0.0011, $p < 0.0001$), but there was no difference between growth rates between treatments (Supplementary Figure 1; GLSLMM: treatment: $t_{276} = 0.6737$, $p = 0.5011$ [“begging” = reference level]).

Tadpoles in both treatments had significantly positive development rates (life stage as a function of age) (GLSLMM: begging: *ß* = 0.1198, *SE* = 0.0076, $p < 0.0001$, no begging: *ß* = 0.1249, *SE* = 0.0076, $p < 0.0001$), but development rates were not different across treatments (Table 2). However, “begging” tadpoles were consistently behind in life stage throughout the entire experiment (Figure 2, Table 2).

Although DSA was not significantly different between treatments throughout tadpole development (all 20 days of body size observation), the minimum DSA values were consistently associated with the experimental treatment, and the maximum DSA values were consistently associated with the control tadpoles. A GLSLMM solely including these minimum and maximum values of body size revealed that the “begging” tadpoles (i.e. minimum DSA values) grew significantly slower than the “no begging” tadpoles (i.e. maximum DSA values) (Supplementary Table 2).

Experiment 3

Tadpoles in both food treatments had significantly positive growth rates (tadpole DSA as a function of age) (GLSLMM: non-supplemented: *ß* = 0.0183, *SE* = 0.0008, $p < 0.0001$, supplemented: *ß* = 0.0222, *SE* = 0.0008, $p < 0.0001$). Since tadpole life stage was positively correlated with age (Pearson’s product-moment correlation: non-supplemented: $t_{478} = 56.2358$, $p < 0.0001$, supplemented: $t_{478} = 73.1882$, $p < 0.0001$), life stage as a covariate of DSA was left out of the GLSLMM testing for the effect of food treatment on DSA. Non-supplemented tadpoles had significantly lower growth rates compared to supplemented tadpoles (Supplementary Figure 2, Supplementary Table 3).

Tadpoles in both food treatments had significantly positive development rates (life stage as a function of age) (GLSLMM: non-supplemented: *ß* = 0.1727, $SE = 0.0063$, $p < 0.0001$, supplemented: *ß* = 0.1845, $SE = 0.0063$, $p < 0.0001$). However, tadpole life stages were not different between treatments throughout development (GLSLMM: $ß_{supplemented - non\text{-}supplemented} = 0.03429$, $SE = 0.3361$, $p = 0.9208$, age × food treatment: *ß* = 0.0118, $SE = 0.0089$, $p = 0.1886$) (Supplementary Figure 3).

Based on initial observations of feeding probability (0: not fed or 1: fed) (Supplementary Figure 4) and feeding frequency (frequency a tadpole was fed by life stage) (Supplementary Figure 5) by tadpole life stage, as well as on model AICs, a quadratic regression relating feeding responses to tadpole life stage improved model parsimony from that generated by a linear regression ($GLMM_{prob}$ AIC = 527.57, $GQMM_{prob}$ AIC = 522.34, Chi-square test: $X^2 = 7.2295$, $p = 0.0072$; $GLMM_{freq}$ AIC = 1102.2, $GQMM_{freq}$ AIC = 1007.2, Chi-square test: $X^2 = 97.01$, $p < 0.0001$). According to the $GQMM_{prob}$, across both food treatments, the probability of feeding significantly followed a quadratic regression with respect to tadpole life stage (Table 3). The $GQMM_{freq}$ indicated that feeding frequency and tadpole life stage also followed this quadratic relationship ($GQMM_{freq}$: stage: $z = 6.615$, $p < 0.0001$, stage^2: $z = -9.712$, $p < 0.0001$) (Supplementary Table 4).

Across all tadpole life stages, the probability of feeding was approximately 86.39% higher for the non-supplemented tadpoles (Figure 3, Table 3). This effect of food treatment marginally depended on tadpole DSA (Table 3) while it was not affected by tadpole life stage

(GQMM$_{\text{prob}}$: food treatment × life stage: z = -0.577, p = 0.5640, food treatment × life stage^2: z = 0.232, p = 0.8163).

Across all tadpole life stages, non-supplemented tadpoles were fed approximately 1.39 more times than their supplemented siblings (GQMM$_{\text{freq}}$: z = -2.010, p = 0.0444) (Supplementary Figure 6, Supplementary Table 4). This effect of food treatment on feeding frequency did not depend on mean tadpole DSA (Supplementary Table 4) nor tadpole life stage (GQMM$_{\text{freq}}$: food treatment × life stage: z = 0.680, p = 0.4960, food treatment × life stage^2: z = -0.330, p = 0.7410).

DISCUSSION

Experiment 1

Begging in *R. imitator* appears to function as a signal of offspring need. Tadpoles in the low food treatment that grew particularly slowly increased their begging effort (i.e. begging time per begging episode) over time, in contrast to tadpoles in the other two treatments (Figure 1). From the beginning of the experiment, these tadpoles consistently remained at relatively earlier life stages; however, as their ontogenetic stages advanced, they accelerated development to a rate exceeding those achieved in the other treatments (Supplementary Table 1). Although these nutrition-limited tadpoles still took longer to reach metamorphosis, their acceleration in development may be indicative of a trade-off between growth and development, where low-food availability may select for tadpoles to develop faster, possibly at the cost of a smaller body size at metamorphosis (i.e. a "develop now, pay later" strategy; Metcalfe and Monaghan 2001).

Did tadpole begging effort reflect long-term and short-term offspring need?

The observation that begging effort significantly increased over time in smaller, less-developed tadpoles suggests that they ultimately used a larger proportion of energy reserves towards begging compared to those that were larger and consistently reached higher developmental stages. Some studies of offspring begging have distinguished between long- and short-term need. For example, Iacovides and Evans (1998) manipulated both short-term deprivation time and long-term condition via differences in long-term diet. A similar study conducted in domestic pigs manipulated short-term need by removing some piglets from their mothers' udders before milk ejection while accounting for observed variation in long-term body condition (residuals of piglet body mass-to-age ratio) (Weary and Fraser 1995). Price et al. (1996) tested for effects of long-term need on begging intensity in yellow-headed blackbirds

(*Xanthocephalus xanthocephalus*) by using chick gender (males are destined to be bigger at time of fledging) and body condition (a measure of fitness) as measures of long-term need.

According to Price et al. (1996), short-term offspring need is the amount of food required to satiate hunger (subject to rapid change), while long-term need is the growth (or development) rate an individual must attain to reach a target body size (or life stage) (e.g. fledging body mass for birds) by the end of the development period and therefore represents the total investment that the individual requires over the remaining time of development. This definition of long-term need is applied to this experiment in the following section.

Begging effort over the course of offspring development appears to signal long-term need

The results from this experiment suggest that begging effort serves as a signal of long-term need. The relatively smaller, less developed tadpoles in the low food treatment increased begging effort as they got older. Thus, begging effort elicited over the course of tadpole development appears to reflect long-term need. Regarding tadpoles in the low food treatment, chronically feeding them low amounts of food (0.005g/wk compared to 0.02g/wk for the medium food treatment and 0.03g/wk for the high food treatment), and/or maintaining relatively longer periods of food deprivation (5d/wk compared to 2d/wk and 1d/wk) week after week may have caused increasing deficits in long-term nutrition as development progressed (as indicated by relatively slower growth rate and consistently lower developmental stage achieved throughout the experiment). Thus, the significant increase in begging effort over time by tadpoles in the low food treatment may have revealed the gradual increase in the total investment (i.e. parental care) required over the remaining course of development for successful development and metamorphosis. On the other hand, the tadpoles in the other two treatments maintained a relatively constant begging effort throughout development, suggesting that their levels of long-term need were either lower or did not change over time.

Assuming that long-term need was indeed signaled by tadpole begging effort as described above, the absence of an increase in begging effort in the medium and high food treatments additionally suggests that time spent begging served as an honest signal of long-term need. Theoretical considerations derived from the handicap principle (Zahavi 1975) posit that for a signaling behavior to prevent dishonest gain, there must be a cost associated with the behavior (Grafen 1990, Zahavi 1997). Regarding Godfray's offspring signaling theory (1991), a cost associated with plastic begging behavior should minimize the likelihood of "runaway escalation" of begging intensity and thereby minimize conflict of interest between the begging individual and its parent(s) (Rodríguez-Gironés et al. 2001, Johnstone and Godfray 2002; but see McCarty 1996, Abrahams and Evans 1999). Since the larger, more developed tadpoles in this experiment did not increase begging effort as they got older, this measure of begging effort may be considered, from a parent's perspective, a non-deceptive and thus reliable signal of long-term need. Possible costs that may keep such a begging response over time at an evolutionarily equilibrium are proposed in the discussion of Experiment 2. Whether tadpole begging effort (observed within a single feeding event or throughout development) can serve as an honest signal of short-term need in *R. imitator* requires further investigation.

Begging effort may signal short-term need

Interestingly, all tadpoles regardless of food treatment elicited some degree of begging. Despite being consistently ahead in life stage and growing faster than those in the low food treatment, tadpoles in the medium and high food treatments still begged upon contact with the adult female. Therefore, food deprivation *per se* may not have been exclusively experienced by tadpoles in the low food treatment. That is, even relatively larger, well-developing tadpoles likely experience levels of hunger between feeding events. To apply Price et al.'s (1996) definition of short-term need, begging by a tadpole at a particular instant may predominantly reflect how much food the tadpole needs to satiate the hunger it has accumulated since the last feed. Given that begging effort was similar across treatments during the early stages of development (Figure 1), it is conceivable that during this time, all tadpoles, regardless of food treatment, experienced similar levels of hunger and thus short-term need.

However, it is worth noting that even on the first day of observation, merely four days into food treatment regimes, tadpoles in the low food treatment were significantly less developed compared to those in the other two treatments. Thus, short-term need appears to have diverged to some extent, despite the fact that differences in tadpole life stage early in the experiment were not reflected in differences in begging effort. To explore the potential for tadpole begging effort to signal short-term need, future experiments involving more strongly differentiated food treatments are recommended. Moreover, experiments should be kept as time-series experiments (ideally throughout the entire tadpole development period) in order to account for the possibility that variable short-term need (or lack thereof) may be accompanied by the presence of variable long-term need.

Was the pattern of begging effort throughout tadpole development affected by food mass alone?

Although tadpole begging time appeared to reflect metabolic energy reserves, follow-up experiments are needed to determine whether differences in tadpole need and hence begging effort were driven by food mass or time of food deprivation, both of which were manipulated to create food treatments. Food treatments were differentiated by the mass of food fed during a feeding event, as well as the specific day(s) those feeding events occurred every week. Tadpoles in the low food treatment were food-deprived six consecutive days a week, those in the medium food treatment were deprived two consecutive days a week, and those in the high food treatment only went one day per week without being fed. These time durations may be considered equivalent to time of food deprivation, a factor that has previously been found to affect offspring begging intensity. For example, in a laboratory experiment, ring-billed gull (*Larus delawarensis*) chicks increased begging call rate, loudness, and pecking rate with food deprivation time (1, 4, and 12 h), and this trend resulted from both high- and low-condition chicks (Iacovides and Evans 1998). A different study in which food was experimentally withheld from a group of nestling barn swallows (*Hirundo rustica*) found that nestlings that were food-deprived for two, 3-hr periods increased syllable duration of begging calls (evidence of increased begging intensity in this species) upon approach of a parent while their non-food-deprived nest mates did not (Sacchi et al. 2002).

In *R. imitator*, if deprivation time indeed affects tadpole body size and life stage, and these parameters accurately reflect tadpole need, tadpoles varying in deprivation time should show significant differences in begging effort (e.g. begging duration per begging episode) regardless of the total amount of food consumed. Yet because both food mass and deprivation time were different across food treatments, it is still unclear which or how much (if both) of food mass or deprivation time drove differences in tadpole need and thereby begging effort. Thus, future experiments that separate the effects of food mass and time of food deprivation on tadpole begging behavior are recommended.

Exploring other parameters for more accurate estimation of begging effort

Finally, variable aspects of *R. imitator* begging behavior not investigated in this experiment should be explored as potential signals of tadpole need. Such aspects may include number or mean duration of begging bouts observed within a single begging episode. The intensity of the vibrational movements during begging appeared to vary across begging episodes. Therefore, in quantifying tadpole begging effort, future studies should explore parameters like vibration rate (e.g. number of back-and-forth vibrations per unit time) and vibration amplitude (e.g. mean arc length traced by nose tip per begging bout). Also, although *R. imitator* tadpoles do not make any sounds or calls when they beg, unlike "begging" offspring in many other taxa, the distinct mouth-gaping behavior accompanied by oblique body posture directed toward a present adult during begging may offer interesting avenues for more accurate estimation of begging effort. For instance, Kilner used "ordinal posture ranks" based on a qualitative scale spanning from zero (no begging) to four (gape open, head vertically back, and neck stretched) to estimate what the study calls "begging intensity" in canaries (*Serinus canaria*) (2001).

Although body movements observed during begging bouts have been characterized in some species (e.g. European magpie [*Pica pica*]; Redondo and Castro 1992), so far, no study has attempted to rigorously quantify them in a non-avian species. Taking a more "micro-scale" approach involving multiple begging parameters may first allow comparisons of begging effort to be more accurate. Secondly, it may allow multimodal offspring signaling to be tested in the species. In theory, more than one parameter of a begging episode (e.g. mouth-gaping rate in a begging episode and begging bout duration) may reflect tadpole need (i.e. multiple signals communicate [or collectively enhance the receiver's perception of] a single message, or different signals communicate individually different messages; see the “backup signal” and “multiple message” hypotheses proposed by Johnstone 1996).

Experiment 2

Although evidence for a begging cost on tadpole growth may be considered preliminary (Supplementary Table 2), begging appeared to involve a development cost to tadpoles.

Evidence for a development cost incurred by tadpole begging

Specifically, tadpoles that were induced to beg were consistently behind in life stage throughout the entire experiment (Figure 2). According to a recent model, parental care can control offspring development rate as a means of optimizing offspring growth and minimizing environmental risks to offspring (Klug and Bonsall 2014). Slow development may be costly for *R. imitator* tadpoles for several reasons. First, slow-developing tadpoles are predicted to metamorphose at small (suboptimal) body sizes (see Experiment 1; reviewed in Newman 1992), which can in turn reduce survival immediately following metamorphosis (an already vulnerable period for anurans Altwegg and Reyer 2003, Chelgren et al. 2006) and later as adults (e.g. Briggs 2008, Camargo et al. 2008). A “freshly” metamorphosed froglet may compensate for low energy reserves (i.e. small size) via high foraging activity but doing so may increase risk of dehydration and predation (reviewed in Tarvin et al. 2015), the latter especially under compromised locomotor performance (Chelgren et al. 2006, Ficetola and De Bernardi 2006).

Environmental risks that are largely inherent in *R. imitator* larval life history should also select against prolonged tadpole development and thus particularly intense begging efforts. The phytotelmata that *R. imitator* tadpoles occupy are low in nutrients and small in volume (usually $\leq$ 25ml; Brown et al. 2010). Assuming that the nutrient shortage in these pools facilitated the evolution of obligate trophic egg-provisioning (thereby female participation in parental care) (Brown et al. 2010), prolonged tadpole development has the potential to decrease fitness across all family members. At any given instant, the small phytotelmata may not contain as many predators as larger pools or ponds occupied by offspring of other anuran species. However, the tadpole phase in *R. imitator* lasts > 2 mos, and each egg-provisioning episode (with both parents present) can last > 3 hrs (M. Yoshioka, personal observation). Although provisioning episodes have been observed to occur during the evening hours, whether the process is an evolved strategy to evade diurnal predators is unknown. Furthermore, tadpole predation rates can be quite high (>50%) in *O. pumilio*, a species of poison frog that also exhibits tadpole development in phytotelmata and trophic egg-feeding (Maple 2002) (albeit without male participation). While the environments that *R. imitator* occupy in Peru are wet or moist year-round, slow-developing tadpoles may face heightened risks of habitat desiccation (a common risk among some phytotelm-breeding poison frogs; e.g. Summers and McKeon 2004). Finally, whether excessive begging interferes with a tadpole's responses to environmental cues that might otherwise trigger fast or accelerated development is worth investigating.

Preliminary evidence for a growth cost incurred by tadpole begging

A statistically significant effect of treatment on tadpole growth rate was found when the absolute minimum and maximum values of body size (corresponding to the "begging" and "no begging" treatment respectively), were considered in the statistical model. Thus, the experiment offers preliminary evidence that begging incurs a cost on tadpole growth rate. Negative effects of begging on offspring body size have been found in some bird species. A study using magpie chicks and a similar experimental setup involving a begging-induced treatment and a control group found that begging-induced chicks grew 8.15% slower than chicks of the control group

(Rodríguez Gironés 2001). Kilner (2001) found that in canaries, a species in which chick body mass starts to decline midway through the nestling period (common for many altricial avian species), chicks with higher postural rank scores (higher begging intensity) experienced greater losses of body mass as they developed. However, according to Chappell and Bachman (2002), "begging is brief compared to most behavioral or physiological events that affect energy metabolism and always occurs against a background of substantial, continuous and often variable energy utilization" (p. 144). In the experiment, the lack of statistically significant effect(s) of begging on raw measures of tadpole body size may be attributed to the limited sample size vulnerable to high variation. Alternatively, the amount of tadpole begging induced by the "begging" treatment may have been too weak to detect a growth cost (see next section).

Whether tadpole begging is costly may depend on environmental context

Detectability of a growth cost may sacrificed by begging tadpoles may increase under more natural conditions. Tadpoles induced to beg were exposed to an adult female for 6 min four times a week, while in a natural trophic egg-feeding event (albeit occurring every ≈ 7 d), a tadpole may be in contact with one or both parents for at least 3.5 hours (M. Yoshioka, personal observation, see Chapter 2). In addition, given that metabolic and physiologic processes may be influenced by an array of environmental factors, it is important to consider that the production of any begging cost may be highly context-dependent. Common grackle (*Quiscalus quiscula*) nestlings have been shown to shorten begging durations with increased sunlight exposure, illustrating that begging constraints can be context dependent, even within a single day of offspring development (Glassey et al. 2014). The tadpoles used in these experiments were held, aside from treatment variables, under constant conditions to provide an otherwise suitable environment for tadpole growth and development. Tadpoles were fed only 0.008g per week less than those in the medium food treatment in Experiment 1. However, phytotelmata are expected to vary in a number of environmental conditions (see above). Therefore, a given cost of begging may only be identified if such factors are allowed to periodically vary or reach levels that are suboptimal for tadpole development. Moreover, a reduced tolerance to environmental stress may in itself represent a cost of begging. Future investigations into such context-dependent constraints on tadpole begging may offer interesting insights.

Exploring alternative or additional costs of tadpole begging

It should not be assumed that one cost, identified empirically, such as the development-related cost previously discussed, is the only cost associated with the behavior. Furthermore, in order to properly detect other alternative or additional costs of tadpole begging may require monitoring of tadpole characteristics that are distinctly different from body size and life stage. Although growth and development costs are primarily tested in studies of offspring begging behavior, more empirical attention has in recent years been given to physiological costs, which do not necessarily manifest as changes in body size or developmental stage (e.g. costs of gas exchange: Chappell and Bachman 1998, Abraham and Evans 1999; food processing: Secor 2008; defenses against oxidative stress: Noguera et al. 2009; immunocompetence: Moreno-Rueda and Redondo 2012).

In future experiments, physiological costs should be tested for not only how they respond to begging expenditure, but also how they may influence tadpole growth and/or development. Simultaneously testing for multiple costs of tadpole begging may allow a distinction to be made between direct costs and indirect costs of begging, the latter of which may be more appropriately considered residual effects of begging costs. Such an approach may not only allow investigation of how costs may interact (e.g. synergistically—additive or multiplicative effects), but also deepen the understanding of physiological mechanisms through which begging costs operate.

Experiment 3

Based on this experiment, trophic egg-provisioning *R. imitator* parents appear to allocate their investment according to offspring energy reserves and thus to offspring need. Throughout the entire course of tadpole development, parents were generally more likely to feed the tadpole that did not receive any supplemental food over its food-supplemented sibling. Consequently, the non-supplemented tadpoles were on average fed relatively more frequently.

Trophic egg-provisioning as a response to offspring need

Statistically, the influence of tadpole body size on feeding differences between food treatments was minimal to absent (Table 3, Supplementary Table 4). This result is likely due to correlation between tadpole body size and life stage, both of which were strongly correlated with tadpole age (see Results: Experiment 3). Consequently, when both tadpole body size and the polynomial terms of life stage were included as model predictors, the GQMMs revealed a weak influence of tadpole body size on the effect of food treatment on egg-feeding. However, while food treatments generated similar developmental patterns in tadpoles (Supplementary Figure 3), they produced significantly different tadpole growth rates, where non-supplemented tadpoles grew significantly slower than supplemented tadpoles (Supplementary Figure 2, Supplementary Table 3). Therefore, parents may have exercised egg-feeding preference for the non-food-supplemented tadpole relative due to differences in their perceived levels of offspring body size (i.e. offspring energy reserves) and hence offspring need.

Findings from this experiment are congruent with the interpretation that begging effort signals offspring need in *R. imitator* (see discussion on Experiment 1). Egg-provisioning was higher for the “needier” (non-supplemented) tadpole relative its food-supplemented sibling, and this feeding pattern was maintained throughout the entire period of tadpole development (Figure 3). Similarly, Atlantic puffin (*Fratercula arctica*) parents were found to reduce provisioning rates when their chicks received supplemental food thereby allowing care to be allocated more efficiently for current and future offspring (Dahl et al. 2005). Given the results from Experiments 1 and 2, it is probable that the non-supplemented tadpoles that grew slower experienced chronically lower nutrition levels relative to the supplemented tadpoles and therefore substantially higher begging effort. Although we cannot confirm that the non-supplemented tadpoles increased their begging efforts over developmental time (similar to the low food treatment in Experiment 1), it is evident that in Experiment 3, parents consistently exercised a feeding priority for the tadpole that signaled higher levels of need relative to its sibling, regardless of tadpole age or life stage. Thus, *R. imitator* parents appear to deliver care according to perceived levels of offspring need (as signaled by begging efforts). Such a pattern may be confirmed by replicating the experiment while also monitoring tadpole begging effort throughout the course of tadpole development. Doing so may also aid in distinguishing between short- and long-term offspring need, as what principally drives differential allocation of parental effort.

That trophic egg-feeding was predicted as a quadratic function of tadpole life stage regardless of food treatment (Figure 3, Supplementary Figure 6) was an unanticipated yet interesting outcome (but see Supplementary Figure 7). The gradual increase in egg-feeding during the first half of tadpole development may be considered additional evidence for parental responsiveness to levels of offspring need, in this case, those varying over offspring development time. Perhaps regardless of a tadpole's nutritional status or body condition, the energy requirements for successful development steadily increase in demand over the earlier stages of tadpole development. During this period, the tadpole not only increases in body size but undergoes a series of dramatic morphological changes, including hindlimb development (critical for jumping locomotion; Marsh 1994) and color pattern emergence (critical for Müllerian mimicry in *R. imitator*; Yeager et al. 2012). Cases with similar temporal patterns of parental investment over the course of offspring-rearing periods have been observed in seabirds. For instance, in pigeon guillemots (*Cepphus columba*) (Emms and Verbeek 1991) and two albatross species (*Thalassarche chrysostoma, T. melanophris*) (Huin et al. 2000), provisioning rates were found to steadily increase as developing chicks got older. Also, similar to the results of this experiment, the seabird studies showed a non-linear relationship between parental effort and offspring age, where provisioning rates began to decline roughly halfway into chick development.

Explaining the decline in egg-feeding during the latter phase of tadpole development

Ranitomeya imitator parents may be selected to decrease egg-feeding once the energetic cost of provisioning begins to exceed the offspring benefit associated with tadpoles continuing to receive trophic eggs (at a consistently increasing or asymptotic rate). Although the experiment held food available to parents constant and thus eliminated environmental effects on parental care, the production of trophic eggs is likely demanding for the female parent. Also, in such a biparental care system involving monogamous mates, not only is the reproductive success of the male parent strongly dependent on the fitness of his female mate, the male parent may likewise undergo substantial metabolic stress having to endure long calling episodes required to induce maternal egg deposition (i.e. interdependence of each parent's reproductive success; Schlomer et al. 2011). Life history theory states that a fundamental trade-off faced by parents is between current and future reproduction, and this especially applies to contexts involving parental care (as reviewed in Schlomer et al. 2011). That is, provisioning parents may run the risk of crossing a threshold of investment or provisioning effort, above which parental resources used in caring for current offspring would increase long-term reproductive success if allocated to future offspring (Trivers 1974, Chisolm 1999). Therefore in this experiment, parents' eventual decrease in egg-feeding for both tadpoles indicates that parents may be selected to minimize their own long-term reproductive costs, as well as those potentially suffered by future offspring.

Secondly, *R. imitator* parents may decrease egg-feeding during the latter stages of offspring development due to the tadpole's corresponding decline in food-assimilated energy required to successfully metamorphose. Following roughly the first half of development, a *R. imitator* tadpole does not exhibit any substantial structure changes other than tail absorption (which immediately precedes metamorphosis). Similar hypotheses have been proposed for several seabirds. For example, Rein et al. (2000) found that chick provisioning sharply declined halfway into the chick-rearing period in grey-headed albatrosses (*Thalassarche chrysostoma*) and attributed the pattern to a change from chick growth of internal organs and skeleton to feather production, the latter of which depends more on fat-stores than food-assimilated energy. However, while many avian species that exhibit close mirroring of provisioning patterns with offspring growth illustrate that provisioning decisions are likely under strict selection for the purposes of nestling fledging (e.g. Sagar and Horning 1998, Wright et al. 2006), tadpoles in this experiment continued to increase in body size in a linear manner despite a reduced rate of egg-feeding towards the tail end of tadpole development. Thus, the experiment lacks any evidence that would otherwise suggest that the non-linear feeding pattern in *R. imitator* reflects optimization of jumping ability of post-metamorphic offspring, conceivably the anuran equivalent of post-fledging flight ability in birds.

Peak in egg-feeding frequency at a morphologically distinct tadpole life stage

Although the statistical analyses strongly supported a negative quadratic function of tadpole life stage on both the probability of feeding and feeding frequency, a closer examination of feeding frequency across individual families indicates that the majority (seven of the 11) of parental pairs fed their offspring the most frequently when tadpoles were at life stage 9 (Figure 4), and not exactly halfway into tadpole development (life stages 6-7), as statistically predicted. More specifically, mean feeding frequency drastically increased at tadpole life stages 8-9 following a brief decrease between stages 6 and 7 (Figure 4).

According to the derivations of life stage values used in the study, tadpole life stage 8 corresponds to Gosner stages 38-39, when hindlimb length stabilizes and individual hindtoes begin to proportionally differentiate (Gosner 1960). Tadpole stage 9 in this study corresponds to Gosner stages 40-41, when pigment patterns become more pronounced, the cloacal tail piece is lost, and forelimb “elbows” emerge (Gosner 1960). Thus, physiological and structural processes that usually occur between Gosner stages 30 and 41 are marked by the development of key traits required for successful metamorphosis. Gosner stages 40 and 41 are characterized by final structural changes of the head and mouth and forelimb emergence, which, for *R. imitator* tadpoles, occur shortly before they enter the terrestrial, “froglet” phase. Thus, presumably in *R. imitator*, energy demands strongly increase in offspring individuals that are on the verge of making this transition from Gosner stages 38-39 to stages 40-41 (stage 8 to 9 in this study). Such a distinct change in offspring need may select parents to increase food supply during this pivotal phase of offspring development. Analogous results have been observed in other taxa. For instance, Mauch and Ricklefs (2005) noticed that in Leach's storm petrels (*Oceanodroma leucorhoa*), meal mass delivered by provisioning parents accelerated during the period when most chicks of the species begin the linear phase of wing growth. Similarly, Huin et al. (2000) found in both grey-headed and black-browed albatrosses (*T. chrysostoma, T. melanophris*), provisioning parents sharply increased foraging-trip duration shortly prior to the fledging phases of their nestlings. A detailed examination of tadpole physiology between Gosner stages 38-39 and stages 40-41 may offer potential explanations for the apparent decrease in offspring need during this time of tadpole development.

Parental investment as a means of balancing a developmental cost of high offspring need

Based on the results from Experiments 1 and 2, a "needy" tadpole (i.e. with low energy reserves) is likely to beg intensely and signal the benefit it would receive from additional provisioning of food. Consequently, the combination of low nutritional status and energy-expensive begging efforts likely results in slow development (see Table 2, Supplementary Table 1). In this experiment, it was expected that the non-supplemented and thus relatively "needier" tadpoles would exhibit a similar outcome. Despite food treatments generating significantly different tadpole growth rates (Supplementary Figure 2) (and perhaps begging efforts), non-supplemented tadpoles generally developed in tandem with their food-supplemented siblings (Supplementary Figure 3). Whether costs incurred by honest begging behavior are balanced or outweighed by the "benefits" of parental care has been difficult to determine (Rodriguez-Girones et al. 1996, Moreno-Rueda 2007). However, a recent theoretical model suggests that parental control over offspring developmental rate can act as a significant benefit of parental care (Klug and Bonsall 2014). In this experiment, more frequent food-provisioning may have "offset" the developmental lag that the non-supplemented tadpoles might have otherwise suffered from reduced body size and/or energetically costly levels of begging. Such balancing of costs may have allowed them to ultimately develop at rates similar to those achieved by the supplemented tadpoles.

For bird species that have been studied in this regard, feeding history does not typically predict fledgling size (e.g. final wing length) of nestlings, and nutrition deficiency often triggers periods of compensatory growth via parental increases in meal size (e.g. Ricklefs and Schew 1994, Granadeiro et al. 2000, Hipfner et al. 2006). This experiment provides evidence for a similar developmental cost of poorer offspring body condition (i.e. higher tadpole need), comparable to the growth cost observed in nutrient-deficient chicks or nestlings. While offspring growth is likely subject to strong selection in birds (for successful fledging), developmental progression through physiological and corresponding morphological changes appears to be under strong selection in *R. imitator* tadpoles. Such a development cost was implicated in Experiment 1, where tadpoles in the low food treatment accelerated development, even exceeding the development rates produced by tadpoles that were relatively better-fed. Thus, a future experiment should explicitly assess how parental investment in *R. imitator* can act to offset a development cost suffered by particularly “needy” tadpoles. Furthermore, since signaling high levels of need may require offspring energy that could otherwise be allocated towards developmental processes (see Experiment 2), the “benefit” a tadpole receives from provisioned food may also be viewed as compensation for begging costs.

CONCLUSION

In summary, by examining offspring begging behavior and parental care in the mimic poison frog, this study offers a novel contribution in an anuran to our understanding of the evolutionary basis of such behaviors. According to the experimental findings, offspring begging behavior elicited by *Ranitomeya imitator* tadpoles reflects nutritional deficits accumulated over developmental time, thereby operating as an honest signal of long-term need. Such signaling efforts are costly, where offspring individuals that beg more strongly take relatively longer to reach developmental marker stages. Reduced growth rate appears to represent an additional cost of begging. Finally, food-provisioning parents appear to exercise a feeding priority for offspring that receive less food and hence have higher offspring need. Because higher offspring need is translated through stronger begging efforts, differential allocation of parental care likely reflects variation in begging efforts (across offspring individuals). The theoretical conditions for offspring begging behavior to remain an evolutionary stable signal through parent-offspring interactions were met by the "single-offspring, single site" system exhibited by the mimic poison frog.

Experiment 3 also revealed interesting aspects of *R. imitator* reproductive life history. First, provisioning parents appear to follow a feeding schedule reflective of energy requirements predicted by distinct stages of tadpole development, independent of tadpole body condition. Such provisioning pattern may also be considered evidence for parental response to offspring need. However, the "need" here appears to be related more directly to physiological requirements of offspring development and thus distinct from the need that is primarily determined by food availability (which affects body size and associated begging efforts). Secondly, energy assimilated from provisioned food has the potential to offset the developmental cost associated with high offspring need and associated begging efforts. Therefore, tadpole progression through developmental stages critical for successful metamorphosis may be under strong selection in this species.

The study demonstrates that in *R. imitator,* offspring begging behavior functions as a context-dependent, reproductively important signal for provisioning parents. To explicitly test if offspring signals are actively interpreted and employed by *R. imitator* parents during care allocation, it should be recognized that parental care in the species is not exclusively observed during events of trophic egg-provisioning. Consistent with the ancestral state of male-only care in neotropical poison frogs (Summers and McKeon 2004), parental care in the mimic poison frog is largely determined by paternal behaviors. The male parent not only directs maternal egg-provisioning; it also transports freshly hatched tadpoles to rearing sites and guards and monitors all offspring individuals throughout the entire development period. Thus, it can be assumed that for any offspring individual, the majority of its parent-offspring interactions involve contact with the male parent. Given this, it may be insightful to test for differences between maternal and parental responses to offspring signals, including those that may be expressed during or prior to the egg-hatching phase. Such investigations may reveal the relative contribution of sex-specific parental roles in determining ultimate reproductive success.

FIGURES AND TABLES

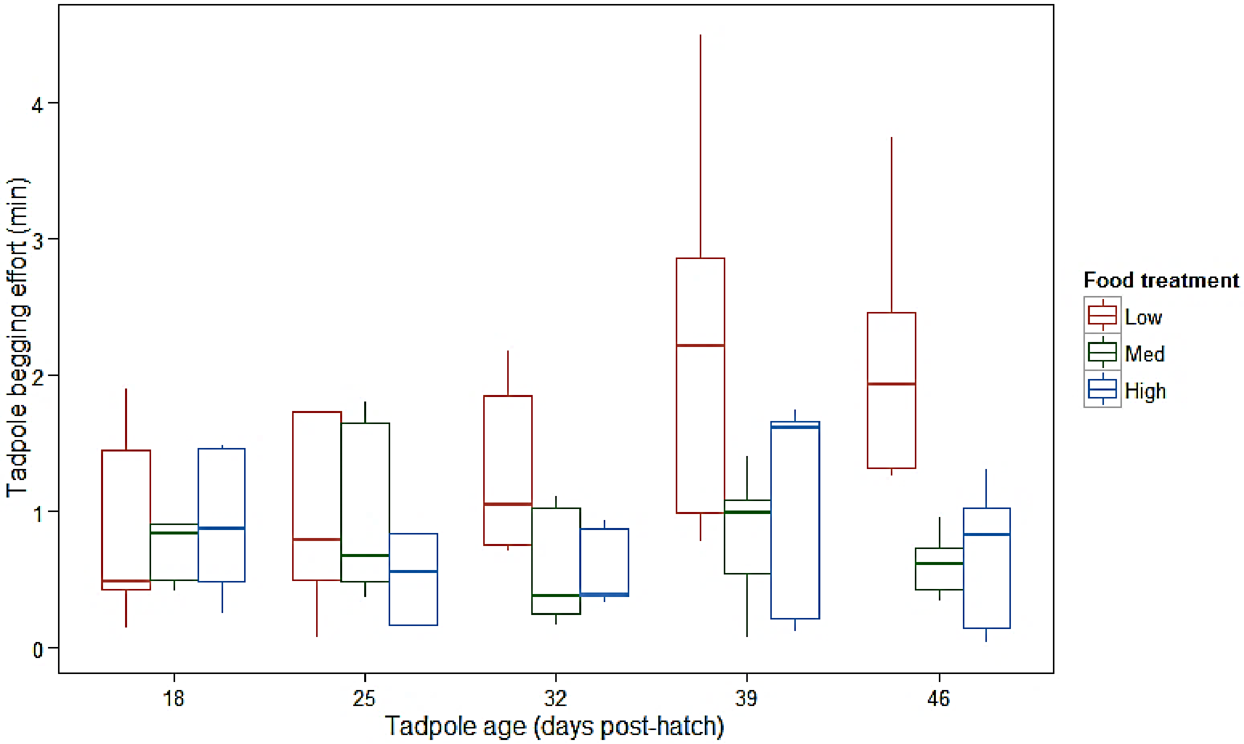

Figure 1 Comparison of tadpole begging effort (total time spent begging during the 6-min duration of contact with an adult female) as a function of tadpole age between food treatments: low (0.005g/week), medium (0.02g/week), and high (0.03g/week) food.
(*n* = 5 tadpoles)

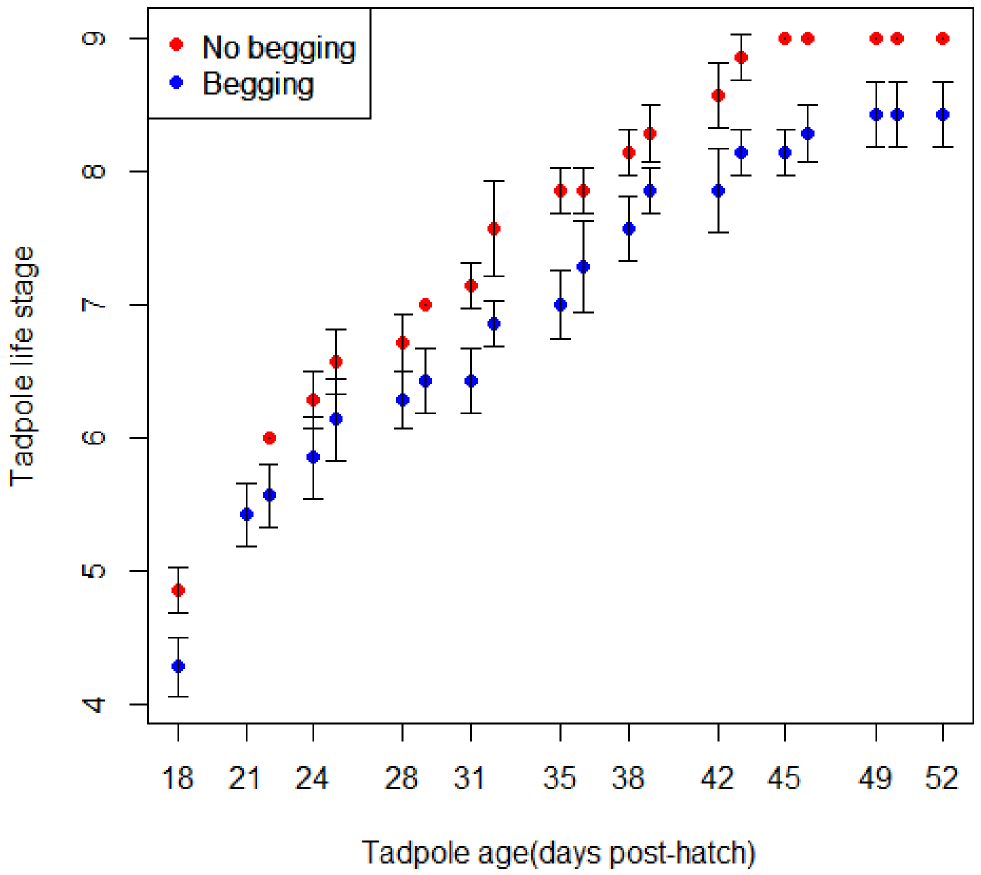

Figure 2 Comparison of tadpole development (mean life stage ± SE as a function of tadpole age) between the “begging (induced to beg) and “no begging” (not induced to beg) treatments. (*n* = 5 tadpoles)

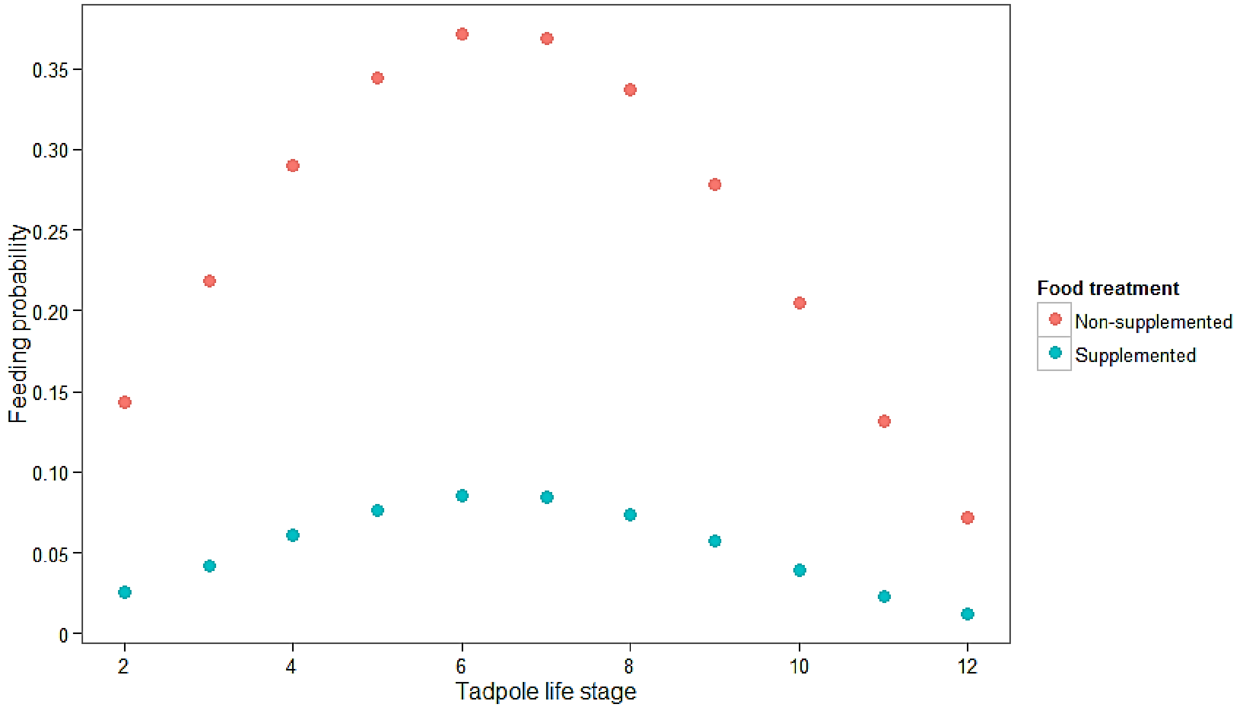

Figure 3 Comparison of feeding probability (generated from binary egg-feeding response [0: not fed, 1: fed]) as a function of tadpole life stage between food treatments: “non-supplemented” and “supplemented”. Feeding probabilities were calculated from log-odd values estimated by all coefficient estimates generated by generalized quadratic mixed effects model with logit link function. (n = 11 tadpole sibling pairs)

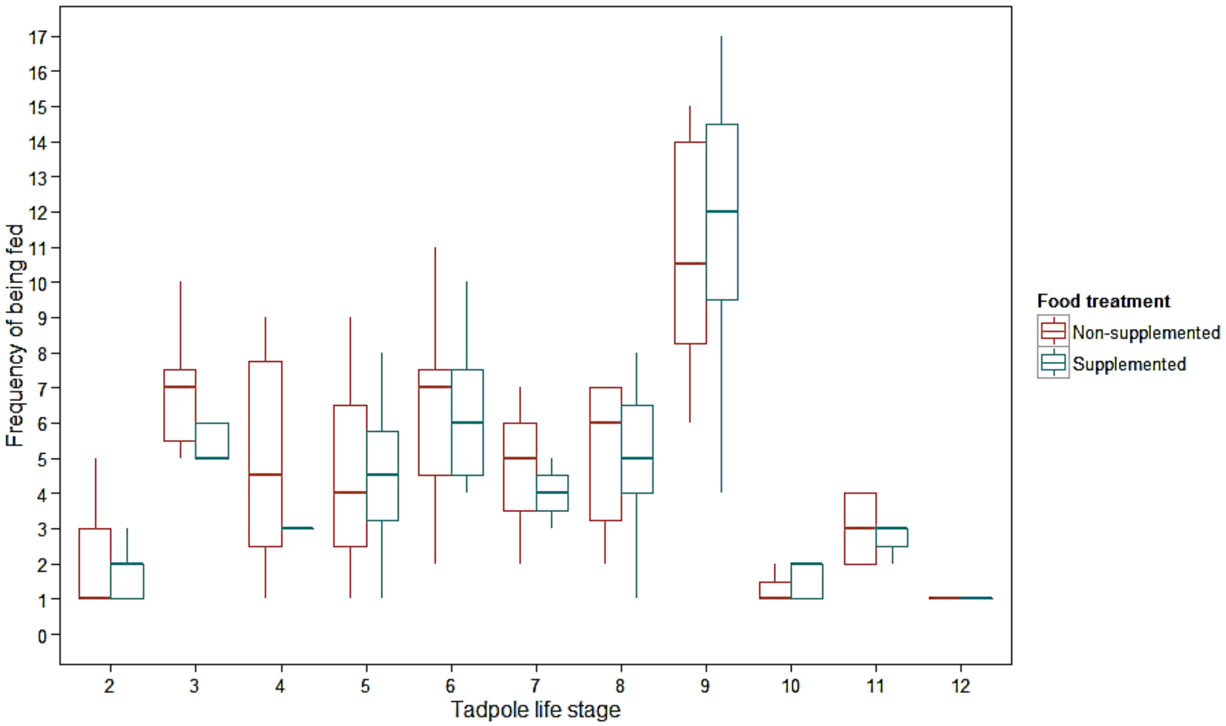

Figure 4 Comparison of mean feeding frequency (number of times a tadpole was fed; frequency of 1: fed in binary egg-feeding response) by tadpole life stage between food treatments: "non-supplemented" and "supplemented". Mean feeding frequencies were calculated across tadpole families. (n = 11 tadpole sibling pairs)

TABLES

Table 1 Results of GLS linear mixed effects model for comparison of tadpole begging time by age between low food and other two food treatments. (Low food treatment is the reference treatment.) *Denotes statistical significance at $\alpha < 0.05$.

Parameter	*ß*	*SE*	*t*	*p*
Intercept	0.2142	0.5815	0.3683	0.7138
Tadpole Age	0.0388	0.0172	2.2511	0.0276 *
Med Food	1.0429	0.8224	1.2680	0.2090
High Food	0.8006	0.8224	0.9734	0.3338
Age × Med Food	-0.0528	0.0244	-2.1691	0.0335 *
Age × High Food	-0.0436	0.0244	-1.7885	0.0781 ·

Correlation structure: AR(1); parameter estimate: Phi=0.1879. (n = 5 tadpoles)

Table 2 Results of GLS linear mixed effects model for comparison of tadpole development (life stage as a function of age) between “begging” and “no begging” treatments, using “begging” treatment as the reference treatment. *Denotes statistical significance at $\alpha < 0.05$.

Parameter	*ß*	*SE*	*t*	*p*
Intercept	3.2894	0.2835	11.6032	<0.0001*
Tadpole Age	0.1198	0.0076	15.7235	<0.0001*
Treatment	0.3909	0.0401	9.7481	<0.0001*
Age × Treatment	0.0051	0.0108	0.4760	0.6344

Correlation structure: AR(1); parameter estimate: Phi=0.6350. (n = 5 tadpoles)

Table 3 Results of generalized quadratic mixed effects model for estimating the effect of tadpole life stage on log-odds of trophic egg-feeding (0: not fed or 1: fed) and comparison of egg-feeding between food treatments (“non-supplemented” and “supplemented”) with tadpole body size (dorsal surface area [cm^2]; “DSA”) as a covariate of food treatment. (“Non-supplemented” treatment is the reference treatment.) *Denotes statistical significance and · denotes marginal statistical significance at $\alpha < 0.05$.

Parameter	*ß*	*SE*	*z*	*p*
Intercept	-3.8449	1.0475	-3.670	0.0002 *
Tadpole life stage	0.7078	0.3491	2.027	0.0426 *
(Tadpole life stage)^2	-0.0654	0.0267	-2.452	0.0142 *
Treatment	-1.8485	0.6995	-2.643	0.0082 *
Tadpole DSA	0.2643	1.0511	0.251	0.8015
Treatment × Tadpole DSA	1.5166	0.8889	1.706	0.0880 ·

(n = 11 tadpole sibling pairs)

CHAPTER 2: Field observations of parent-offspring interactions during a presumed trophic egg-feeding event recorded in Tarapoto, Peru (July 2014)

INTRODUCTION

Although previous research on anuran communication focused mainly on the adult life stage, especially in the context of courtship and mating (Ryan 2001), increasing attention has been paid to signaling during early life history stages (e.g. egg stage: Warkentin 2005, tadpole stage: Kiesecker et al. 1996, Parris et al. 2006, Stynoski and Noble 2012). These recent studies of communication modes in earlier life stages have mainly focused on the acquisition of signals or cues important for predator evasion. However, growing research on maternal egg-provisioning in tropical anurans has revealed that particularly in systems of biparental care, developing offspring can signal during parent-offspring interactions *(Colostenthus beebei*: Bourne et al. 2001; *Dendrobates vanzolinii*: Caldwell and Oliveira 1999; *Anotheca spinosa*: Jungfer and Weygoldt 1999; *Chirixalus eiffingeri*: Kam and Yang 2002; *Oophaga pumilio*: Stynoski and Noble 2012). Additionally, communication (via male vocalization) is observed between parents during coordination of offspring food-provisioning, and thus outside the traditional, intra-sexual context of mating and courtship (*Dendrobates vanzolinii*: Caldwell and Oliveira 1999, *Colostenthus beebei*: Bourne et al. 2001).

In the mimic poison frog (*Ranitomeya imitator*), biparental care involves offspring attendance and coordination of maternal egg-provisioning by the male parent. In this species native to northeastern Peru, tadpoles are deposited individually in small, nutrient-poor phytotelmata, where they complete development with nutrition derived almost exclusively from maternally-provided trophic (unfertilized) eggs. Roughly every 7 d of tadpole development, the male stimulates maternal, trophic-egg deposition by calling, during which the tadpole produces rapid body vibrations (i.e. offspring begging behavior) directed toward the approaching or attending parent (Brown et al. 2008). To date, these offspring feeding events have been either described in general terms as above (Brown et al. 2008) or monitored discontinuously (i.e. monitoring signs of egg-feeding and offspring growth post-male removal; Tumulty et al. 2013). Together, these studies have provided evidence consistent with the hypothesis that offspring growth and development in the mimic poison frog is highly dependent on the male orchestration of maternal egg-provisioning (Tumulty et al. 2013). However, for biparental care to optimize long-term reproductive success, such intra-familial networks of communication must involve the transmission and use of honest and thus reliable signals (Zahavi 1975), a concept that has commonly been tested in avian systems (Budden and Wright, 2001).

Chapter 1 demonstrated that in mimic poison frogs, tadpole begging effort honestly represents offspring need as predicted by nutritional level and also, that egg-provisioning is allocated preferentially to tadpoles of higher need. In addition to providing "real-world" context for the laboratory experiments reported in Chapter 1, observing natural egg-provisioning events in the field allows for a deeper understanding of the signaling mechanisms employed by each family member during these events. Furthermore, the methods used here allowed for a finely detailed description of familial interactions and associated behaviors on a chronologically continuous-time scale.

The video footage also reveals details of offspring signaling and egg-provisioning in *R. imitator* that likely would remain unknown in controlled, laboratory settings. First, this event began in the late afternoon (after 1500 h Peru Standard Time [PET]) and lasted for over 4 h. Secondly, it appears that tadpole begging behavior is not exclusively triggered by physical contact with either the tadpole pool or the tadpole itself by the parent. That is, the tadpole may be sensitive to a combination of visual, chemical, auditory, and/or tactile cues, ultimately triggering the onset of tadpole begging behavior. Also, the duration and intensity of male calling episodes appear to be affected by maternal behavior and environmental conditions (light availability is discussed). The latter factor may also affect male proximity to the tadpole, specifically if low light availability causes vocalizing males to re-locate away from the site of egg-provisioning (possibly to minimize predation risk on behalf of its offspring and female partner). Although such speculations remain unconfirmed, they open interesting avenues for more empirically rigorous investigations into *R. imitator* family dynamics.

A total of six video records captured parent-offspring interactions in *Ranitomeya imitator* between early- and mid-July of 2014 in lowland Tarapoto, Peru. All videos were recorded at separate sites, offering glimpses into six separate sets of parents caring for their own individual tadpoles. Each video caught interactions between parents and one tadpole offspring, as well as interactions between the male and female parent. Upon review of all videos, one video was found with the female parent and tadpole only in the field of view, although the male was confirmed to be present via auditory cues. In order to characterize the series of events involved in biparental care in *R. imitator*, the video that captured the longest episode of parent-offspring contact (almost the total 4 hr of recording time) was chosen for chronological narration.

METHODS

Offspring tadpoles were individually deposited naturally by the male parent in clear, rainwater-filled, plastic-cups secured near axils of *Diffienbachia spp*. located in marked male territories. The clear, plastic containers each had a top diameter of 4 cm, enabling visualization of parent-offspring interactions, which likely would have been impossible to observe in a natural plant axil (axil openings usually have diameters < 1.5 cm). Videos were recorded using GoPro Hero3 White Edition cameras (GoPro, Inc., San Mateo, CA), each attached to a GoPro Battery Backpack. Both camera components were housed in a GoPro waterproof housing to minimize exposure to humidity and rain. Four, 55-mm diopter-filter (macro) lenses (with 1, 2, 4, and 10× magnification) (Digital Concepts, Inc., St. Louis, MO) attached to a macro lens adapter were fixed with elastic bands to the waterproof housing and positioned directly in front of the camera lens for image magnification. When not in use, these lenses were always stored exposed to air so as to prevent fogging when employed in the field. Upon preparation for video-recording, the camera was secured to a wooden post with miniature bungee cords and positioned 3 cm above the tadpole container with the lenses facing perpendicular to the water surface. The camera and the supplemental battery together supplied enough power to record approximately 4 hr of video at 1280 × 720 pixel resolution and 60 frames per second. This resolution was chosen as it offered the longest recording duration without substantially compensating visibility of tadpole behaviors.

RESULTS AND DISCUSSION

Narrative description of parent-offspring interactions observed during a presumed trophic egg-feeding event recorded between hours 1440 and 1830 on July 4, 2014:

The video indicates first signs of male attendance at approximately 1500 h PET and continues to record behaviors of all three family members through the onset of dawn, which is then followed by further reduction of light availability through the end of the video. Supplying enough recording power for a total video length of 4 hrs, the video-camera battery depletes at 1830 h, at which point, the field of view is difficult to decipher due to evening darkness. However, the reflection of the tadpole pool is faintly detectable through the end of footage and due to the continuation of male calls and ripple movements indicating tadpole begging, it is assumed that both parents are still present in relatively close proximity to the tadpole through the end of footage. Although the eventual trophic egg-deposition by the female is not captured by the video, it was confirmed to occur at some time after 1830 h, as two fresh, trophic eggs were observed in the tadpole pool the following morning at 0900 h.

Video narration

At approximately 1500 h PET, the male approaches the tadpole pool, perches above the pool water, and looks down at the tadpole, which starts making vibrational begging movements with its head in the direction of the perched male. Short durations of begging are observed while the male continues to have his gaze directed toward the tadpole. The male then starts calling after perching above the tadpole water for roughly 38 mins. The calling bouts consist of 4-6 monosyllabic chirps with 1-sec intervals. After the male calls for about 3 mins (continuous chirps collectively constitute a “calling episode” hereafter), the female begins to move closer to the tadpole pool and calling male, the latter of which now shifts his gaze to his female mate and begins to decrease the duration of pausing intervals between chirps. The tadpole still continues to exhibit short begging bouts. After reaching the tadpole pool, the female positions herself directly above the water surface of the tadpole pool. Roughly 30 sec after the female's approach, the tadpole exhibits its first high-intensity begging bout characterized by very intense vibrations made with its body directed toward the female.

At 1542 h, after roughly 2 min into her initial approach, the female's posterior makes physical contact with the tadpole water and faces her head opposite the tadpole. Begging bouts of mixed vibrational intensity, bout length, and resting interval duration continue throughout the remainder of video footage. Approximately 10 min after the female's first touch with the tadpole water, the male ceases his calling episode lasting for roughly 15 mins. For the next 20 mins, the female stays motionless, still "sitting" at the tadpole pool, barely touching the water. At 1555 h, roughly 13 mins after initial female-water contact, the male shifts his position so that his head is now facing opposite the tadpole water and his body laterally opposite that of the female. At this time, the male starts calling again and although calling episodes continue to be exhibited for the next 2 hrs, the episodes are much shorter in duration (33 mins-1 hr for particularly short episodes and 2 – 4 mins for longer episodes) compared to the initial episode (roughly 15 mins) that began before female approach to the tadpole pool.

At 1613 h, the female positions her body slightly above the water, so that it is no longer in contact with the tadpole water. After the female remains in this position for 20 mins with her head still facing opposite the tadpole, the male changes the direction of his head and gaze toward the female, a behavior that is immediately followed by a calling episode lasting about 2.5 mins. Shortly thereafter, the male repositions his head to face opposite the tadpole and his gaze away from the female, while making his initial contact with the tadpole water. Meanwhile, the female stays stationary, still directly above the tadpole water surface. The tadpole is still eliciting begging bouts with pauses between bouts lasting no more than 1 min. At this point, begging bouts now appear to be directly triggered by parental movements independent of physical contact with the tadpole water.

After 1.25 hrs of the female in a fixed position just above the tadpole water surface, she begins to adjust her body at 1726 and 1730 h, both parents submerge half of their bodies and “sit” in the tadpole water, facing their heads opposite the tadpole. At 1737 h, the onset of dawn is visible due to a clear decrease in sunlight in the field of view. With the exception of some minor adjustments with the body, both parents remain in physical contact with the tadpole water. The tadpole continues to beg and the male continues to call periodically.

At 1748 h, the male suddenly jumps out of the tadpole pool and lands somewhere outside the field of view. However, the male continues to call without noticeably changing sound frequency of chirps. Following this escape from the field of view, the male begins and maintains a single, long-lived calling episode involving chirps with a noticeably lower sound pitch. Chirps are produced once again with 1-sec pausing intervals. Observed through the end of the video, this long calling episode lasts at least 39 mins.

For the remainder of the feeding event, the female adjusts her body in the tadpole water every 1 – 3 mins without substantial changes in head or gaze direction. The tadpole still begs periodically, and the male continues to call with steady, low-pitched chirps. At 1807 h, the field of view has become restricted to the faint reflection of the tadpole water surface. However, slightly visible ripple movements of the tadpole water suggest that the female is still in contact with the tadpole, inducing it to continue begging through the end of footage. As mentioned earlier, although the video does not capture the actual event of trophic egg-deposition by the female parent, it is assumed that the event occurs at some time after 1830 h (the time when the footage cuts out due to depletion of battery power). At 0900 h the following morning, the occurrence of maternal egg-deposition was confirmed by the observation of two trophic eggs present in the tadpole pool.

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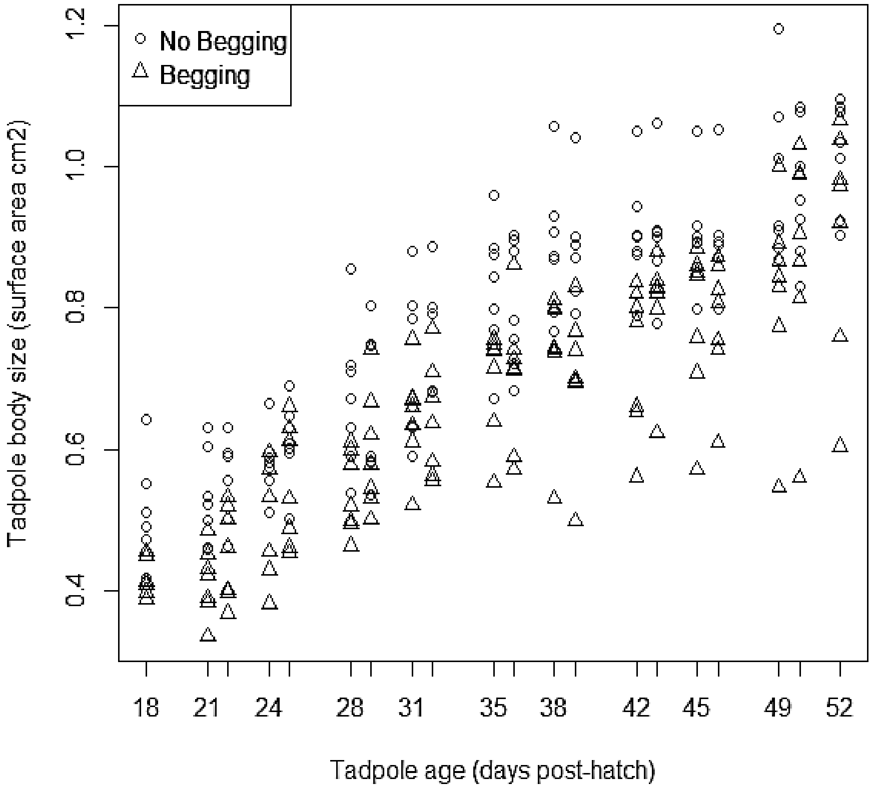
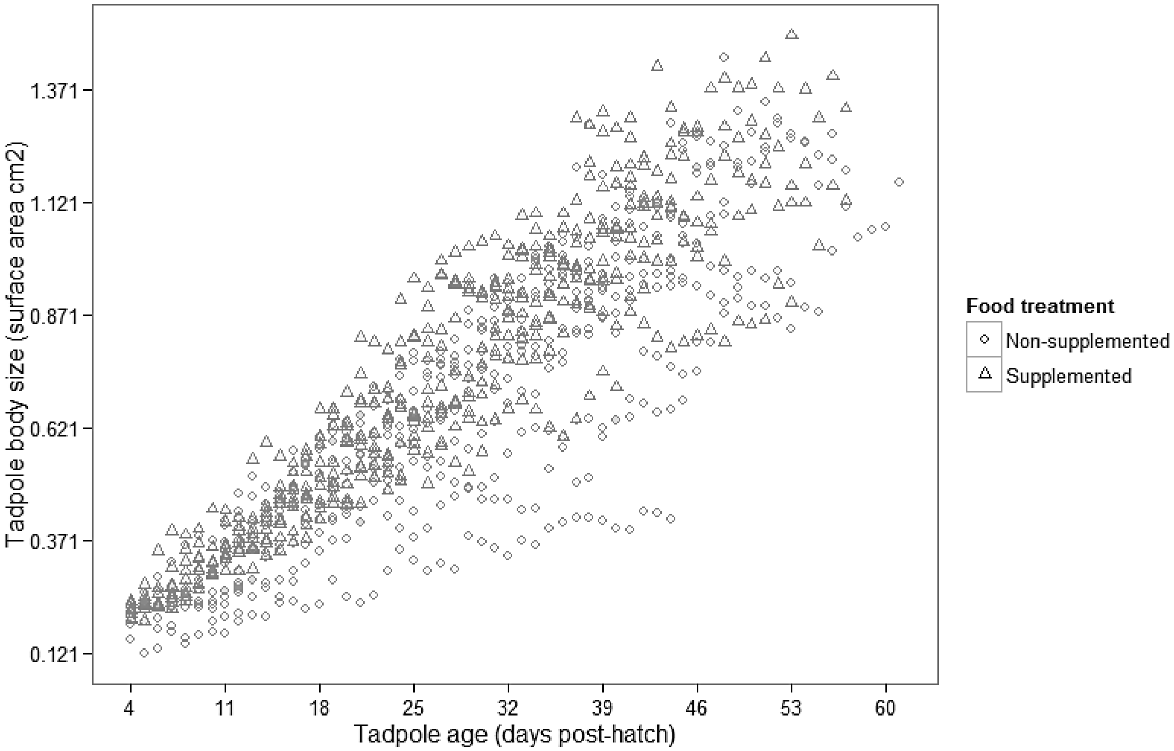
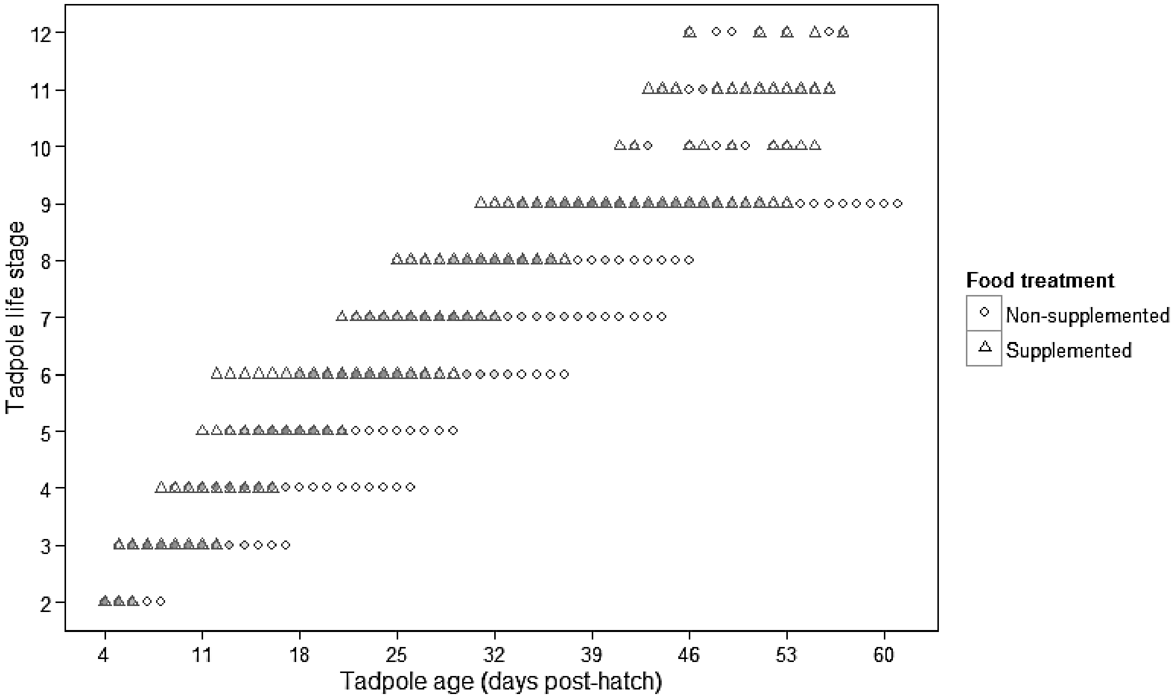
SUPPLEMENTARY FIGURES AND TABLES



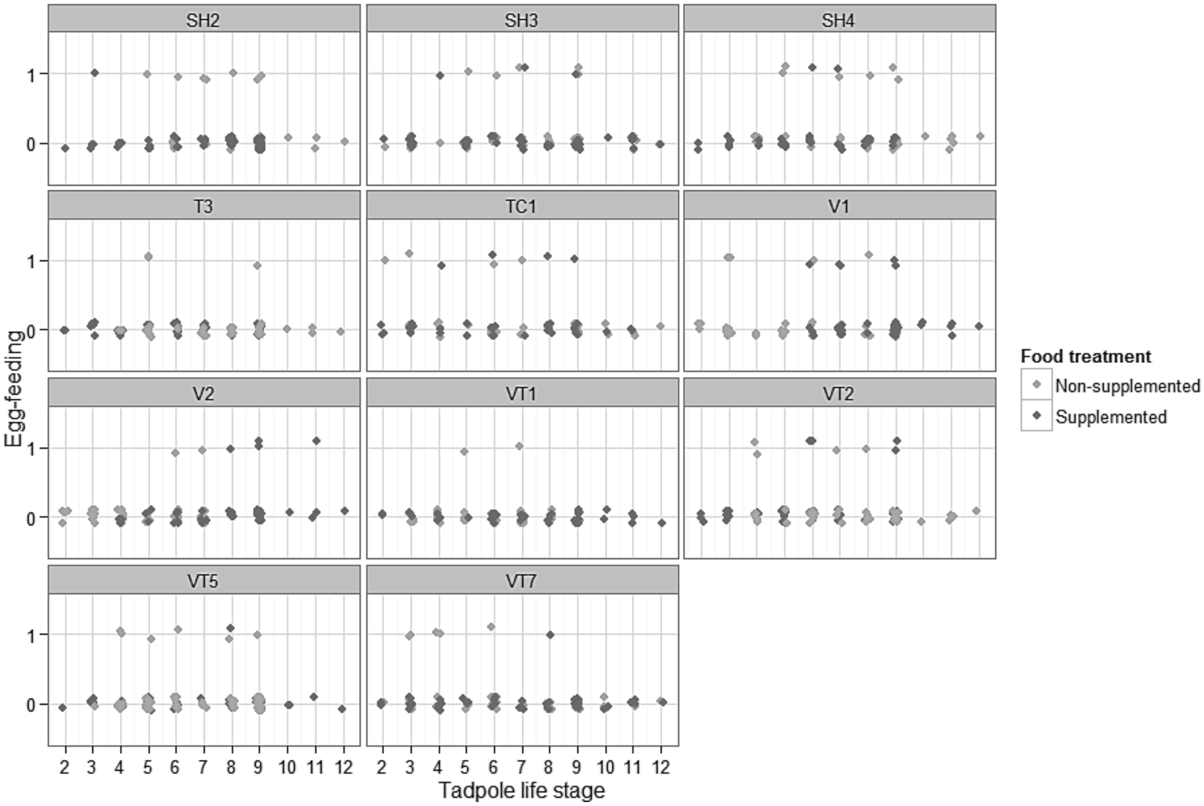
Supplementary Figure 1 - Tadpole growth (body size as a function of tadpole age) is compared between treatments: “No Begging” and “Begging”. (*n* = 5 tadpoles)



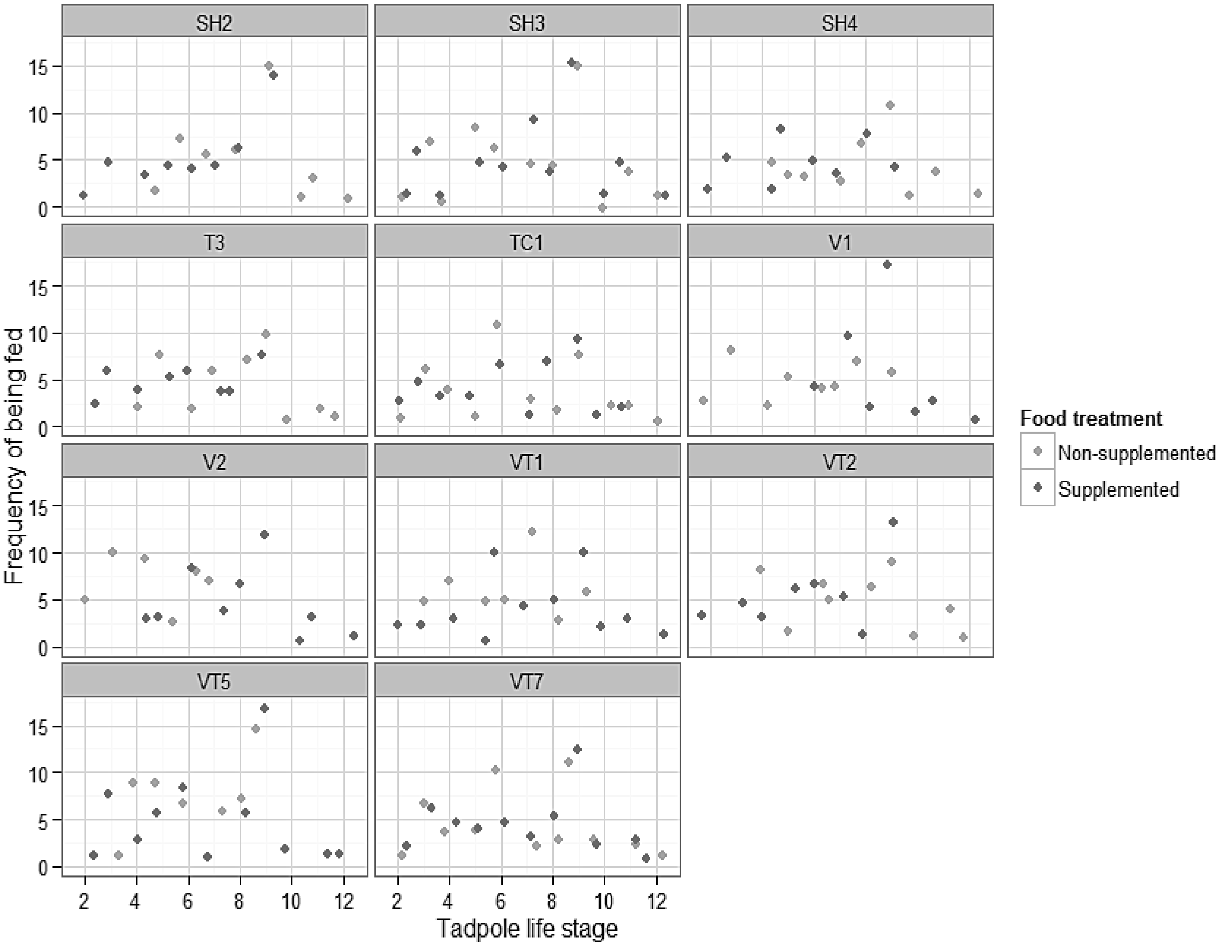
Supplementary Figure 2 - Tadpole growth (body size as a function of tadpole age) is compared between food treatments: "Non-supplemented" and "Supplemented". (*n* = 11 tadpole sibling pairs)



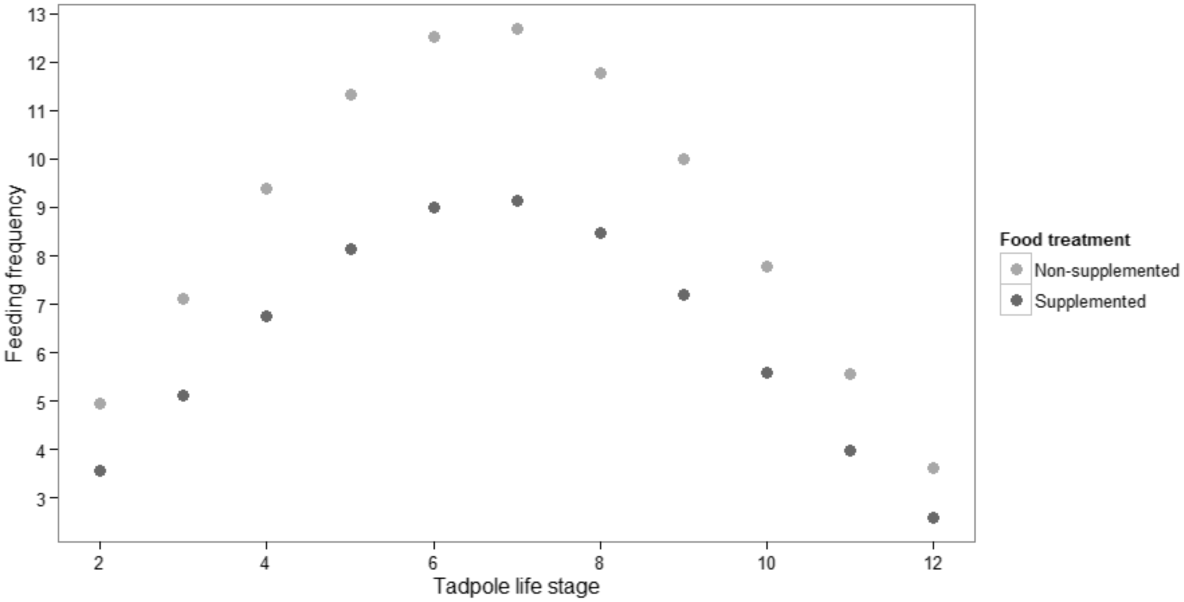
Supplementary Figure 3 - Tadpole development (life stage as a function of tadpole age) is compared between food treatments: "Non-supplemented" and "Supplemented". (*n* = 11 tadpole sibling pairs)



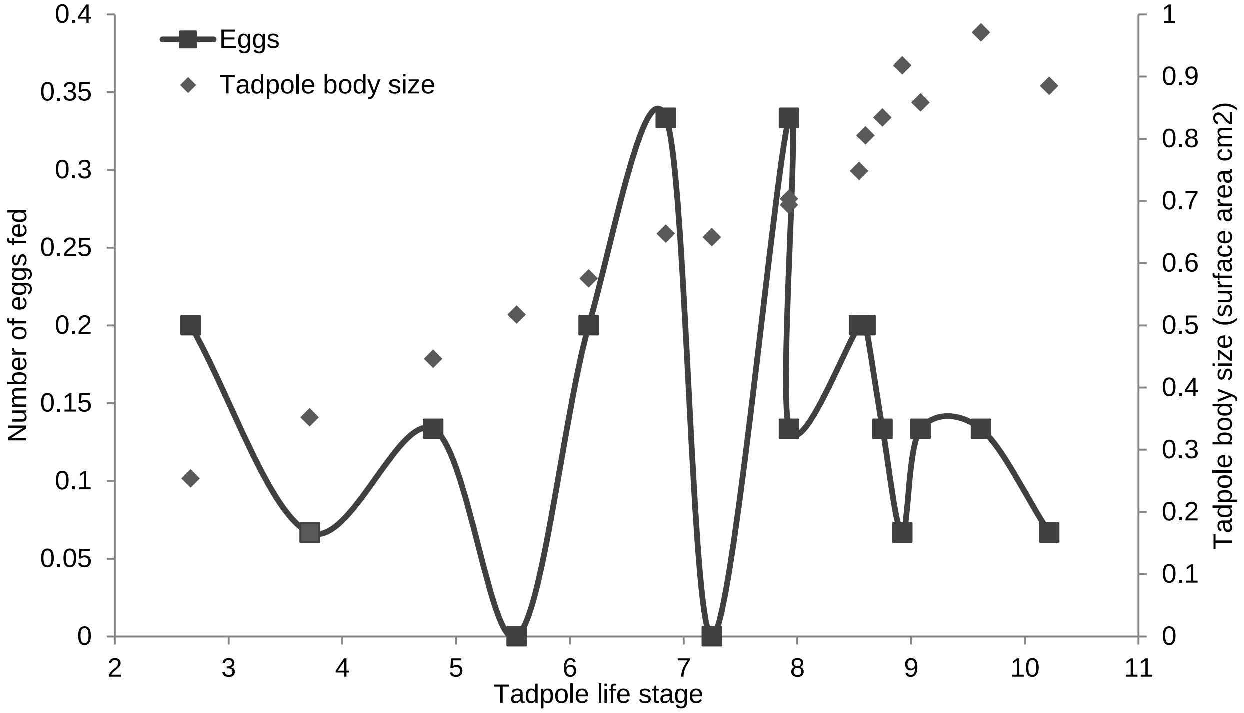
Supplementary Figure 4 Feeding probability (0: not fed or 1: fed) by tadpole life stage is compared between food treatments: "Non-supplemented" and "Supplemented". Codes above plots denote tadpole families where each family consisted of two sibling tadpoles, each subjected to either food treatment. Overlapping points are slightly "jittered" around shared values. (*n* = 11 tadpole sibling pairs)



Supplementary Figure 5 Feeding frequency (number of times a tadpole was fed; frequency of 1: fed in binary egg-feeding response) by tadpole life stage is compared between food treatments: **"Non-supplemented" and "Supplemented".** Codes above plots denote tadpole families where each family consisted of two sibling tadpoles, each subjected to either food treatment. Overlapping points are slightly "jittered" around shared values. (*n* = 11 tadpole sibling pairs)



Supplementary Figure 6 – Feeding frequency (number of times a tadpole is fed; frequency of 1: fed in binary egg-feeding response) as a function of tadpole life stage is compared between food treatments: “Non-supplemented” and “Supplemented”. Feeding frequencies were calculated from log-count values estimated by all coefficient estimates generated by generalized quadratic mixed effects model with log link function. (*n* = 11 tadpole sibling pairs)



Supplementary Figure 7 – Displayed are number of eggs fed per tadpole and tadpole body size as a function of tadpole life stage during preliminary field observations in Tarapoto, Peru (May – July 2014). For ease of interpretation, all values for each tadpole were averaged over 3-d segments from daily observations. (*n* = 5 tadpoles)

Supplementary Table 1 - Results of generalized least squares (GLS) linear mixed effects model for comparison of tadpole development (life stage as a function of age) between low food and other two food treatments. (Low food treatment is the reference treatment.) *Denotes statistical significance and · denotes marginal statistical significance at $\alpha < 0.05$.

Parameter	*ß*	*SE*	*t*	*p*
Intercept	2.4659	0.4752	5.1888	< 0.0001*
Tadpole Age	0.1429	0.0140	10.2321	< 0.0001*
Med Food	2.3569	0.6721	3.5069	0.0008*
High Food	3.0310	0.6721	4.5100	< 0.0001*
Age × Med Food	-0.0393	0.0197	-1.9920	0.0503 ·
Age × High Food	-0.0500	0.0197	-2.5277	0.0138*

Correlation structure: AR(1); parameter estimate: Phi=0.3102. (*n* = 5 tadpoles)

Supplementary Table 2 - Results of GLS linear mixed effects model for comparison of tadpole growth (body size as a function of age) between treatments, using minimum and maximum values of tadpole body size (dorsal surface area [cm^2]). ("Begging" treatment [which produced minimum values] is the reference treatment.) *Denotes statistical significance at $\alpha < 0.05$.

Parameter	*ß*	*SE*	*t*	*p*
Intercept	0.3415	0.0540	6.3290	<0.0001*
Tadpole Age	0.0161	0.0015	11.0765	<0.0001*
Treatment	0.1036	0.0760	1.3634	0.1812
Age × Treatment	0.0084	0.0021	4.0573	0.0003*

Correlation structure: AR(1); parameter estimate: Phi=0.3698. (*n* = 5 tadpoles)

Supplementary Table 3 - Results of GLS linear mixed effects model for comparison of tadpole growth (body size as a function of age) between food treatments, using “non-supplemented” treatment as the reference treatment.) *Denotes statistical significance at $\alpha < 0.05$.

Parameter	*ß*	*SE*	*t*	*p*
Intercept	0.1343	0.0437	3.0731	0.0022 *
Tadpole Age	0.01827	0.0008	22.0711	< 0.0001 *
Treatment	-0.0161	0.0439	-0.3665	0.7216
Age × Treatment	0.0039	0.0012	3.3284	0.0009 *

Correlation structure: AR(1); parameter estimate: Phi=0.8324. (*n* = 11 tadpole sibling pairs)

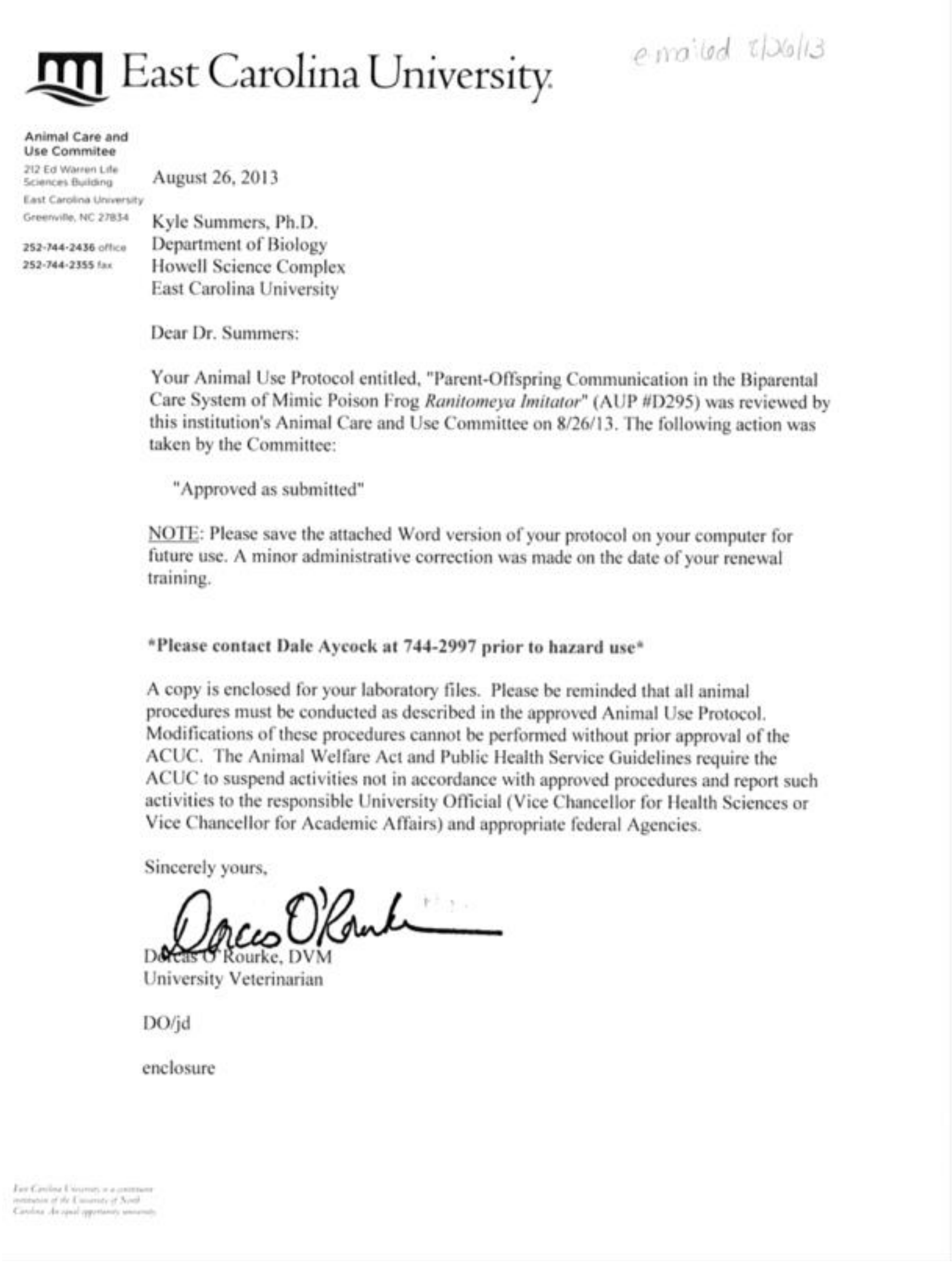
Supplementary Table 4 - Results of generalized quadratic mixed effects model for estimating the effect of tadpole life stage on log-counts of feeding frequency (number of trophic egg-feeding episodes) and comparison of feeding frequency between food treatments (“non-supplemented” and “supplemented”) with tadpole body size (dorsal surface area [cm^2]; “DSA”) as a covariate of food treatment. (“Non-supplemented” treatment is the reference treatment.) *Denotes statistical significance at $\alpha < 0.05$.

Parameter	*ß*	*SE*	*z*	*p*
Intercept	0.09203	0.2375	0.387	0.6984
Tadpole life stage	0.4955	0.0749	6.615	< 0.0001 *
(Tadpole life stage)^2	-0.0439	0.0048	9.712	< 0.0001 *
Treatment	-0.3297	0.1640	-2.010	0.0444 *
Tadpole DSA	0.7066	0.3473	2.034	0.0419 *
Treatment × Tadpole DSA	0.3487	0.2169	1.608	0.1079

(*n* = 11 tadpole sibling pairs)

APPENDIX: Institutional Animal Care and Use Committee approval letters

Approval for laboratory experiments



East Carolina University.

e-mailed 8/26/13

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834
252-744-2436 office
252-744-2355 fax

August 26, 2013

Kyle Summers, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Summers:

Your Animal Use Protocol entitled, "Parent-Offspring Communication in the Biparental Care System of Mimic Poison Frog *Ranitomeya Imitator*" (AUP #D295) was reviewed by this institution's Animal Care and Use Committee on 8/26/13. The following action was taken by the Committee:

"Approved as submitted"

NOTE: Please save the attached Word version of your protocol on your computer for future use. A minor administrative correction was made on the date of your renewal training.

Please contact Dale Aycock at 744-2997 prior to hazard use

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

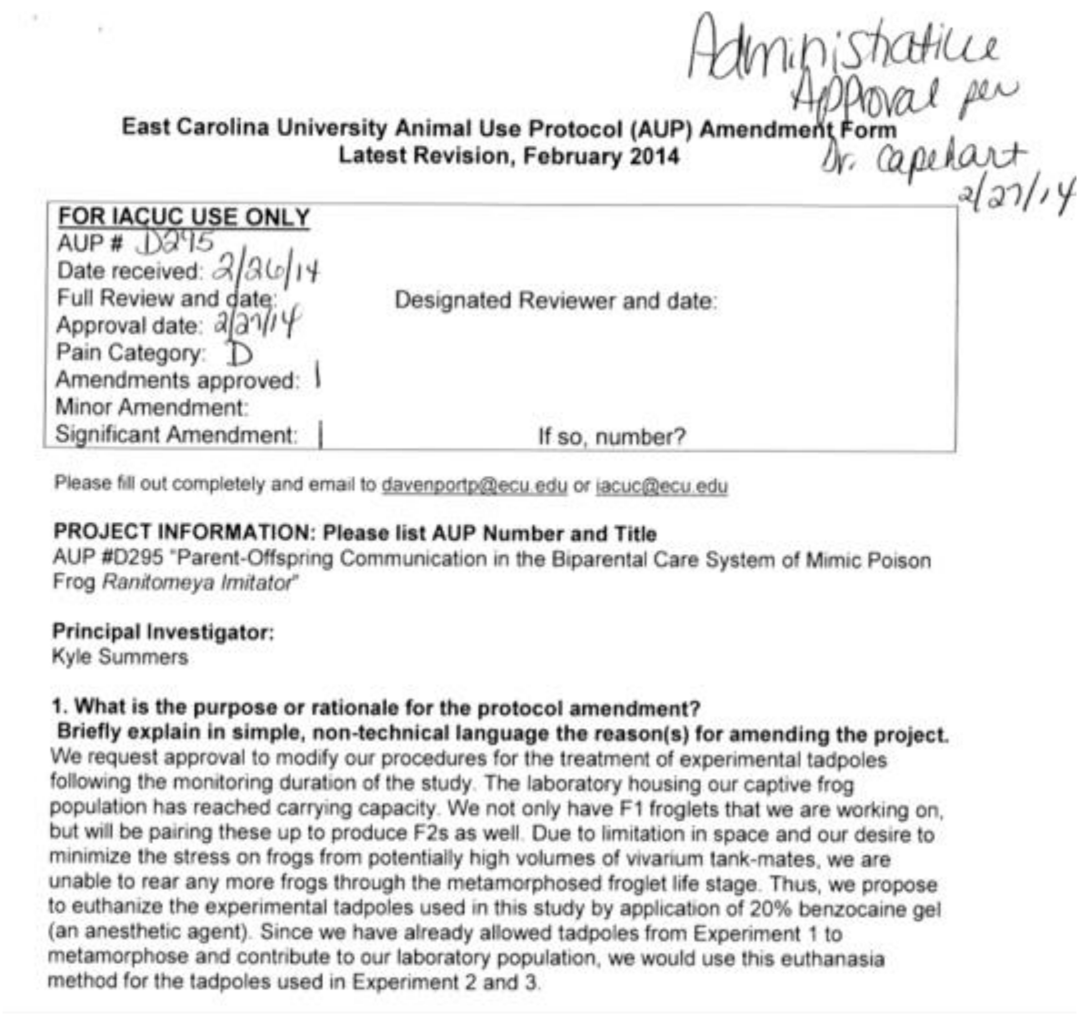
Sincerely yours,

Dorcas O'Rourke

Dorcas O'Rourke, DVM
University Veterinarian

DO/jd

enclosure



Administrative Approval per Dr. Capehart 2/27/14

East Carolina University Animal Use Protocol (AUP) Amendment Form
Latest Revision, February 2014

FOR IACUC USE ONLY	
AUP # D295	
Date received: 2/26/14	
Full Review and date:	Designated Reviewer and date:
Approval date: 2/27/14	
Pain Category: D	
Amendments approved: 1	
Minor Amendment:	
Significant Amendment: 1	If so, number?

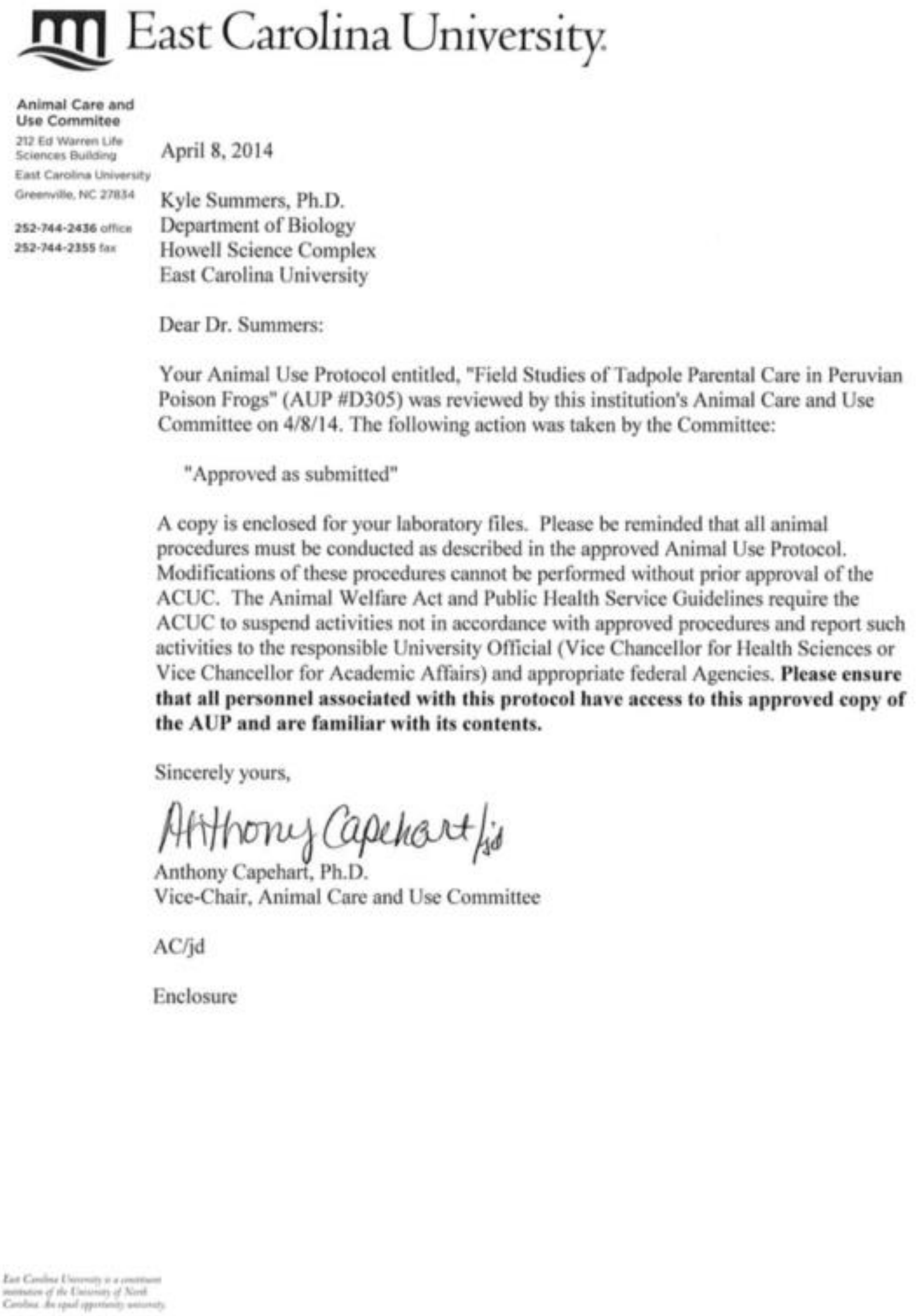
Please fill out completely and email to davenportp@ecu.edu or iacuc@ecu.edu

PROJECT INFORMATION: Please list AUP Number and Title
AUP #D295 "Parent-Offspring Communication in the Biparental Care System of Mimic Poison Frog *Ranitomeya Imitator*"

Principal Investigator:
Kyle Summers

1. What is the purpose or rationale for the protocol amendment?
Briefly explain in simple, non-technical language the reason(s) for amending the project.
We request approval to modify our procedures for the treatment of experimental tadpoles following the monitoring duration of the study. The laboratory housing our captive frog population has reached carrying capacity. We not only have F1 froglets that we are working on, but will be pairing these up to produce F2s as well. Due to limitation in space and our desire to minimize the stress on frogs from potentially high volumes of vivarium tank-mates, we are unable to rear any more frogs through the metamorphosed froglet life stage. Thus, we propose to euthanize the experimental tadpoles used in this study by application of 20% benzocaine gel (an anesthetic agent). Since we have already allowed tadpoles from Experiment 1 to metamorphose and contribute to our laboratory population, we would use this euthanasia method for the tadpoles used in Experiment 2 and 3.

Approval for field observations



East Carolina University.

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

April 8, 2014

Kyle Summers, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Summers:

Your Animal Use Protocol entitled, "Field Studies of Tadpole Parental Care in Peruvian Poison Frogs" (AUP #D305) was reviewed by this institution's Animal Care and Use Committee on 4/8/14. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP and are familiar with its contents.**

Sincerely yours,

Anthony Capehart/jd

Anthony Capehart, Ph.D.
Vice-Chair, Animal Care and Use Committee

AC/jd

Enclosure

East Carolina University is a constituent institution of the University of North Carolina. An equal opportunity university.