Tadpole begging reveals high quality

M. B. DUGAS*,†, S. A. STRICKLER†‡ & J. L. STYNOSKI§

*Department of Biology, Case Western Reserve University, Cleveland, OH, USA
†Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA
‡Department of Biology, John Carroll University, University Heights, OH, USA
§Department of Biology, Colorado State University, Fort Collins, CO, USA

Keywords:
- begging
- parental care
- parent–offspring communication
- trophic egg

Abstract

Parents can benefit from allocating limited resources nonrandomly among offspring, and offspring solicitation (i.e. begging) is often hypothesized to evolve because it contains information valuable to choosy parents. We tested the predictions of three ‘honest begging’ hypotheses – Signal of Need, Signal of Quality and Signal of Hunger – in the tadpoles of a terrestrial frog (Oophaga pumilio). In this frog, mothers provision tadpoles with trophic eggs, and when mothers visit, tadpoles perform a putative begging signal by stiffening their bodies and vibrating rapidly. We assessed the information content of intense tadpole begging with an experimental manipulation of tadpole condition (need/quality) and food deprivation (hunger). This experiment revealed patterns consistent with the Signal of Quality hypothesis and directly counter to predictions of Signal of Need and Signal of Hunger. Begging effort and performance were higher in more developed and higher condition tadpoles and declined with food deprivation. Free-living mothers were unlikely to feed tadpoles of a nonbegging species experimentally cross-fostered with their own, and allocated larger meals to more developed tadpoles and those that vibrated at higher speed. Mother O. pumilio favour their high-quality young, and because their concurrent offspring are reared in separate nurseries, must do so by making active allocation decisions. Our results suggest that these maternal choices are based at least in part on offspring signals, indicating that offspring solicitation can evolve to signal high quality.

Introduction

Parental and offspring fitness can be maximized at different levels of parental investment (Trivers, 1974). Because developing embryos and free-living offspring often exert some control over how much care they receive, a parent that provides post-zygotic investment risks being forced or manipulated into a suboptimal allocation of effort (Trivers, 1974; Parker et al., 2002; Crespi & Semeniuk, 2004). However, extending care beyond the egg stage also affords a parent the opportunity to adjust investment in response to any shifts in the costs and benefits of delivering care and/or allocating it to a particular offspring (Temme, 1986; Haig, 1990; Mock & Forbes, 1995; Davis et al., 1999). The information required for a parent to make real-time updates to its investment strategy might come from the environment, a parent’s assessment of its own condition, and/or a parent’s assessment of the returns offspring can offer on further investment (Temme, 1986; Haig, 1990; Bateson, 1994; Mock & Forbes, 1995; Forbes & Mock, 1997; Davis et al., 1999). Evaluations of offspring could be made using readily available cues (Maynard Smith & Harper, 2003) like body size, but valuable information might also be unavailable to parents using cues alone (e.g. about immune function: Saino et al., 2000). The value of such cryptic information is hypothesized to drive the evolution of offspring solicitation signals, most often called ‘begging’ (Godfray, 1991; Mock et al., 2011).

The suggestion that offspring begging has evolved to communicate valuable cryptic information has been formalized in three ‘honest begging’ hypotheses (Mock et al., 2011; Caro et al., 2016a). Most popular has been
Signal of Need, the hypothesis that offspring solicitation intensity reflects the marginal value a unit of care offers to offspring fitness (Godfray, 1991, 1995). Based on the assumption that these marginal gains are highest at low fitness values, the key prediction of Signal of Need is that offspring with the lowest current reproductive values (i.e. those that need the most further investment before becoming viable: Clark, 2002) will solicit most intensely (Godfray, 1991, 1995). The Signal of Quality hypothesis predicts the opposite, that offspring in the best condition produce the most intense signals, advertising their past successes and worthiness for further investment (Grafen, 1990; Saino et al., 2000). Signal of Need and Signal of Quality rest on the same explicit assumption that offspring begging contains information about fitness prospects, differing in the direction of the predicted association between this current potential and signal intensity (and implicit assumptions about what type of favouritism maximizes parental fitness: Mock et al., 2011; Caro et al., 2016a,b). The Signal of Hunger hypothesis, on the other hand, does not require that offspring signals reveal reproductive value, instead predicting that signal intensity tracks food deprivation (often called ‘short-term need’: Price et al., 1996; Clark, 2002), with an individual signalling more intensely as its digestive tract empties (Mock et al., 2011). Signal of Hunger and Signal of Need overlap to the extent that gut fullness is, ceteris paribus, predictive of reproductive value, but individuals at all levels of condition can have full and empty guts (Price et al., 1996; Clark, 2002; Wright et al., 2010).

Empirical evidence consistent with Signal of Hunger is common, especially in birds (Mock et al., 2011; Caro et al., 2016a). Unique evidence for Signal of Need (i.e. reproductive value estimated in ways other than manipulating gut fullness) is less convincing, with a recent meta-analysis revealing considerable among-species variation in the extent to which avian begging reveals need (Caro et al., 2016b). Tests of Signal of Quality have been relatively rare in the begging literature (Mock et al., 2011; Caro et al., 2016b). Examples of ‘begging’ traits expressed most intensely by high-quality individuals include colourful plumage (Ligon & Hill, 2010) and soft parts (Saino et al., 2000) in birds and volatile chemical signals in earwigs (Mas et al., 2009). Whether Signal of Quality could be a general explanation for the evolution of begging remains a matter of debate, with Johnstone & Kilner (2011), for example, noting that, ‘it is puzzling why parents would need to intervene actively in the process of food allocation in order to favour offspring in better condition, as SoQ suggests … they could simply allow sibling competition to take its course (p 918)’. In multi-offspring nurseries, the potential for competition among siblings (physical or otherwise) can also make it difficult to distinguish active signalling generally from alternative mechanisms shaping food allocation (Parker et al., 2002; Royle et al., 2002). Here, we tested the function of offspring solicitation signals using a model system in which the potential for direct sibling competition is absent, forcing parents to rely on individual assessments of offspring-generated information to express favouritism.

Female strawberry poison frogs (Oophaga pumilio) deposit one to four newly hatched tadpoles individually in rearing sites (usually water-filled leaf axils), and then, every few days for the next 6 weeks, provision each tadpole with the trophic eggs upon which it is entirely dependent (Brust, 1993; Maple, 2002; Dugas et al., 2016a). When a mother visits, her tadpole performs a likely begging analog, stiffening its body and vibrating rapidly in a manner distinct from swimming (Weygoldt, 1980; Video S1). Because she rears her concurrent offspring in physical isolation, an O. pumilio mother interested in biasing resources towards any subset of her brood must make active choices to do so. Mothers of both this and another poison frog do indeed distribute trophic eggs nonrandomly within multidotadpole broods (Dugas et al., 2016a; Yoshioka et al., 2016), but the extent to which mothers could and do use putative begging displays (rapid vibration) to make these decisions is still unclear.

We tested diagnostic predictions of the Signal of Need, Signal of Quality and Signal of Hunger hypotheses in O. pumilio tadpole begging. We experimentally manipulated tadpole need/quality using a rearing treatment that influenced body condition. If tadpole begging signals need, tadpoles in high condition should beg less intensely, as should more developed tadpoles, as both require less further maternal investment to reach independence (Morey & Reznick, 2000; Dugas et al., 2016b; Yoshioka et al., 2016). If tadpole begging signals quality, high condition and more developed tadpoles should instead beg more intensely. We paired this manipulation of need/quality with a manipulation of hunger, effected with a period of food deprivation matching natural intermeal intervals (Maple, 2002). This allowed us to test the nonexclusive prediction that tadpoles would beg more intensely when hungry (i.e. following food deprivation). Because an experimental manipulation of O. pumilio begging effort proved impractical (see Results), we tested the prediction that begging displays bring about maternal feeding by temporarily swapping the tadpoles of wild O. pumilio mothers with larvae of a different, nonbegging, frog. Finally, we observed maternal visits in the wild to test the prediction that mothers allocate trophic eggs nonrandomly with respect to the intensity of begging performed by their young.

**Materials and methods**

**Study animals**

In May–July 2013, we collected O. pumilio tadpoles from the abandoned Huertos plantations at La Selva...
Biological Station, Costa Rica, searching both natural leaf axils and artificial deposition sites (small plastic cups) established in these plots for several years (Stynoski, 2009). During and after the experiment, we fed these ex situ tadpoles with yolks from eggs of a locally abundant tree frog, Agalychnis callidryas; this alternative food source is readily accepted by O. pumilio tadpoles and is sufficient for normal development (Stynoski et al., 2014). Following the completion of the experiment described below, we reared tadpoles and released metamorphs in collection plots.

**Laboratory manipulation of tadpole hunger, need and quality**

Immediately after collecting tadpoles (day 0), we scored developmental stage (Gosner, 1960) under a dissecting microscope and photographed each tadpole with a size standard for morphology measurements that could be used to monitor growth. In a pilot sample of 24 tadpoles photographed twice each, snout–vent length (SVL) was repeatable (sensu Lessells & Boag, 1987; \( r = 0.95, F_{1,23} = 37.8, P < 0.001 \)) and correlated with two other measures of body size (total length: \( r = 0.954, n = 24, P < 0.001 \); maximum body width: \( r = 0.914, n = 24, P < 0.001 \)). After staging and photographing tadpoles, we moved each to an individual rearing tank filled with 25 mL of rainwater.

Central to this experiment was the independent manipulation of hunger (gut fullness or ‘short-term need’) and long-term fitness prospects (individual quality). To accomplish this, we reared tadpoles under two levels of food availability; the ‘high-food’ treatment was intended to produce high-quality tadpoles and approximated natural provisioning of one egg per day (Brust, 1993; Maple, 2002). The ‘low-food’ treatment was intended to produce low quality (i.e. high need) tadpoles, and we provisioned these at half the ‘high’ rate (0.5 egg per day). To approximate natural feeding patterns (Maple, 2002), we offered high-food tadpoles two eggs on days 1, 3, 5 and 7 post-collection, and offered low-food tadpoles two eggs on days 1 and 5 only. On the morning of day 9, when both groups were due for a meal, we again staged (Gosner, 1960) and photographed each tadpole before transferring it to an observation arena (described below). To standardize hunger between treatment groups, we fed each tadpole ad libitum for the next 10 h. We initially offered two eggs, offering an additional one each hour if eggs had been consumed. Before dark (1700 h), we made sure each tadpole had at least one egg, which we removed at first light (0500 h) the next day (day 10) if uneaten. We recorded the number of yolks each tadpole consumed during ad libitum feeding. We assayed begging behaviour first on post-treatment day 10, the morning after tadpoles had been fed ad libitum. We then food-deprived tadpoles for 48 h (mean natural interval between female visits: Maple, 2002; Stynoski, 2012) and re-assayed begging behaviour (day 12). Following this second begging assay, we again fed tadpoles ad libitum.

Overall survivorship of hand-reared tadpoles was comparable to survivorship in the wild (~ 33%: Maple, 2002). We obtained begging samples from 19 satiated tadpoles reared in the high-food treatment and 16 reared in the low-food treatment. Of these, 17 high-food-reared and all low-food-reared tadpoles survived to the second, food-deprived, assay. One individual in the high-food treatment failed to beg in the satiated trial, and one from the low-food treatment did not beg in the food-deprived treatment; we treated these as missing data because tadpole response is our only means of knowing whether the stimulus female was detected (tadpoles sometimes hid in a corner of the arena). We also mistakenly recorded at an unusable frame-rate on one observation of a satiated high-food tadpole and scored all behaviours except vibration speed from this recording.

**Laboratory assays of begging behaviour**

Begging arenas were square transparent acrylic tanks (4 × 4 × 6.4 cm) in which we placed small styrofoam inserts to reduce the useable space to ~1.5 cm × 1.5 cm × 3 cm, approximating a large leaf axil (Maple, 2002). We stimulated tadpoles to beg by trapping an unrelated female (captured from another plot) inside the arena (sensu Stynoski, 2012; Yoshioka et al., 2016) and recorded interactions from the side using a camera recording at 120 fps (Sony HDR-AS20); empirical work suggests that tadpoles are unlikely to distinguish mothers from other adult frogs (Stynoski & Noble, 2012). We recorded begging assays for 10 min after the tadpole first begged, following previously reported maternal visit duration in this population (Stynoski, 2012). However, our subsequent field observations indicated that females spend only ~80 s in the nursery before laying trophic eggs (see Results). Because we were primarily interested in information mothers might use to make decisions about whether and how much to provision, we scored only the first 80 s of activity after first begging from these trials (≈320 s of high-speed video played at normal playback speed for scoring). Stimulus females never laid trophic eggs.

Although observational work suggests that mothers do not feed tadpoles that do not beg (Stynoski, 2012), we had no a priori information about what elements of begging mothers might use to make provisioning decisions. Following previous work in poison frogs (Stynoski, 2012; Yoshioka et al., 2016), we quantified the time during which tadpoles vibrated with their body held rigid (i.e. begged: Video S1 and S2). Because begging appears to be energetically costly (Stynoski, 2012; Yoshioka et al., 2016), the time an individual spends...
performing this motor pattern seems plausibly tied to its motivation and/or ability to do so. To capture variation in the performance of begging displays, we quantified two other metrics: i) the mean duration of begging bouts (sustained vibration without swimming or inactivity) and ii) the mean speed at which tadpoles vibrated (times the tadpole completed an entire side-to-side body undulation per video frame). We quantified vibration speed during three bouts per trial, the first, last and middle scorable bout; not all bouts were appropriate for scoring because tadpoles were often partially hidden from view by stimulus females and/or oriented in a way that made counting undulations difficult. Given the energetic demands of tadpole locomotion, these two metrics seem plausible measures of physical performance (Wassersug & Sperry, 1977).

Observations of maternal provisioning in the wild and experimental manipulation of tadpole stimulus

We observed maternal provisioning behaviour in the wild in October 2013. With regular censuses, we located tadpoles deposited in artificial rearing sites. On the tree trunk above the cup, we affixed a waterproof video camera (Sony HDR-AS20), set to record at normal speed (30 fps) because high-speed recording requires higher ambient light than is available under the canopy. Most (90%) of maternal visits in this population occur between 0600 and 1100 h (Stynoski, 2012), so we began recordings as soon after first light as was logistically feasible (~0530 h). Camera batteries lasted ~3.5 h, and so whenever possible given equipment availability, we replaced batteries and recorded tadpoles until ~1200 h. We typically had fewer cameras than available tadpoles; because mothers return every ~2 days (Maple, 2002; Stynoski, 2012), we prioritized observations of tadpoles that we had not seen fed by mothers on the prior day. We used females’ idiosyncratic colour and pattern to identify mothers on nonfeeding visits and to identify females caring for more than one tadpole we monitored (Hasse & Pröhl, 2002).

From recordings of maternal visits, we quantified the time females were in physical contact with the artificial rearing cup and the subset of this time during which her cloaca was in the water. We determined whether a female had laid eggs from videos and direct observations when we collected cameras (if eggs were visible, we counted them and looked for the jelly capsules that remain after yolks are consumed: Maple, 2002). In videos, we often saw eggs as they were laid, but other times the eggs were only visible after the mother exited the rearing site. We quantified the same metrics of tadpole behaviour as we had from laboratory trials (total time begging, mean bout duration and vibration frequency in the first, middle and last scorable visit). However, because visits varied in duration, we used the proportion of the visit (mother in the water) a tadpole begged in subsequent analyses rather than total time begging.

In all, we observed 33 visits that 17 unique O. pumilio mothers made to 18 tadpoles. During one visit, the tadpole never begged, one did not complete a bout before the mother exited the rearing cup, and for 1, no bouts were appropriate for scoring vibration speed. We observed trophic egg feeding on 25 visits and were confident that no eggs were laid on six visits. In the remaining two visits, we were unsure of the outcome based on videos (we saw no eggs but saw tadpole behaviour that looked like feeding) and so excluded them from analysis of maternal behaviour.

When we began this study, we expected (based on previous work) that we could experimentally reduce begging effort in wild tadpoles via supplemental feedings (Stynoski, 2012), but this proved not to be the case (see Results). In an effort to address experimentally whether tadpole begging elicits female trophic egg provisioning despite this constraint, we temporarily replaced resident O. pumilio tadpoles with newly hatched (and similarly sized) A. callidryas tadpoles. These substitute tadpoles are sometimes active but do not perform vibrational begging displays (Video S3). We substituted tadpoles only during filming, moving O. pumilio tadpoles to small plastic containers filled with rainwater. After filming, we returned all residents to their rearing sites and released A. callidryas tadpoles in the pond from which clutches were collected. We only swapped resident tadpoles being cared for by females from which we had already recorded provisioning behaviour.

Statistical analyses

We began by assessing the effectiveness of our condition and hunger manipulations. We first compared the high- and low-food treatment groups’ change in SVL and developmental stage (Gosner, 1960) during the 9-day experimental rearing. We used repeated-measures general linear models (PROC GLM; SAS 9.2; SAS Institute, Inc., Cary, NC, USA) for each dependent variable (see Results). In an effort to address experimentally whether tadpole begging elicits female trophic egg provisioning despite this constraint, we temporarily replaced resident O. pumilio tadpoles with newly hatched (and similarly sized) A. callidryas tadpoles. These substitute tadpoles are sometimes active but do not perform vibrational begging displays (Video S3). We substituted tadpoles only during filming, moving O. pumilio tadpoles to small plastic containers filled with rainwater. After filming, we returned all residents to their rearing sites and released A. callidryas tadpoles in the pond from which clutches were collected. We only swapped resident tadpoles being cared for by females from which we had already recorded provisioning behaviour.
high-food tadpoles, and (ii) after the food-deprivation trial, when food deprivation was equivalent for both groups. For these comparisons of yolk consumption, we used generalized linear models (PROC GENMOD) with a negative binomial error distribution to model ‘yolks consumed’ with rearing treatment as a fixed factor and tadpole developmental stage as a covariate. Sample sizes for the analyses described above reflect one individual we failed to stage at collection, two we staged but did not re-photograph after experimental rearing and two trials in which we did not record yolk consumption.

We compared the behaviour of tadpoles from high- and low-food treatments while satiated and food-deprived using linear mixed models (PROC MIXED). We ran separate models for three dependent variables (i) total time begging during the trial (s), (ii) mean begging bout duration (s), and (iii) vibration speed (complete undulations/video frame), and included the fixed effects of rearing treatment (high- or low-food), hunger (satiated or food-deprived), the treatment × hunger interaction and developmental stage. We initially tested the quadratic effect of developmental stage in each model (Stynoski, 2012), but this term was always nonsignificant (all \( P > 0.6 \)) and we thus removed it for clarity. We also tested the prediction that begging performance would decline within trials, as would be expected if this is a physically demanding task, using linear mixed models in which we included the performance metric (vibration frequency or bout duration) as the dependent variable and the fixed effects of treatment, hunger, developmental stage, within-trial bout position (first, middle, last) and the treatment × bout position interaction. In all these models, we included the random subject effect of individual with compound symmetry covariance structure, and retained nonsignificant interactions because we had a priori predictions for each (Whittingham et al., 2006).

We began our analyses of maternal visits to tadpoles in the wild using nonparametric Spearman’s correlations to examine the relationship between developmental stage (Gosner, 1960) and begging behaviour, an analysis that tests the assumption that patterns revealed in laboratory trials mirror those in free-living subjects. We then tested the prediction that maternal provisioning would be associated with tadpole developmental stage and begging behaviour. We began with a generalized linear mixed model with the number of eggs provisioned (range: 0–5) as the dependent variable, the fixed effects of tadpole developmental stage, proportion of time begging, mean begging bout duration, and mean vibration speed, and the random effect of mother ID. With a Poisson error distribution, these data were underdispersed (chi-square/d.f. = 0.75), and alternative error distributions and link functions did not improve model fit. In this model, the random effect of mother ID was estimated as 0, and so we instead used a generalized linear model in which we could apply the scale parameter Pearson’s chi-square/d.f to parameter estimates (PSCALE option). The results of this approach were similar to those of a linear mixed model in which the random effect of mother ID was retained (this model met the assumption of residual normality). Finally, we used a Fisher’s exact test to compare the proportion of visits during which mothers provisioned their own tadpoles to the proportion of visits during which they fed the nonbegging *A. callidryas* tadpoles with which we experimentally replaced them. We only compared female visits to *A. callidryas* tadpoles to our first recorded visits of each mother to her own tadpole; because we reduced sampling effort after first observing a female feed her young, nonfeeding events are likely under-represented in later samples.

### Results

**Laboratory manipulation of tadpole condition and hunger**

Although tadpoles in both rearing treatments grew in SVL during experimental rearing (time: \( F_{1,31} = 4.93, P = 0.034 \)), tadpoles in the high-food treatment grew more (time × treatment interaction: \( F_{1,31} = 4.58, P = 0.040 \); Fig. 1) as did tadpoles that were at later developmental stages at the start of the experiment (stage-at-capture × time: \( F_{1,31} = 12.42, P = 0.001 \)). All tadpoles developed during experimental rearing \( (F_{1,32} = 89.67, P < 0.001) \); Fig. 1), and tadpoles in both treatments progressed through a similar number of stages (treatment × time: \( F_{1,31} = 0.22, P = 0.645 \); Fig. 1). It follows, then, that tadpoles from the high-food treatment became larger for their developmental stage, and indeed, the post-treatment residuals of SVL regressed on stage were higher for tadpoles in the high-food treatment than in the low-food treatment \( (t_{2.1} = 2.16, P = 0.038 \); Fig. 1). When offered food *ad libitum* prior to the first assay of begging, tadpoles from the low-food rearing treatment ate more *A. callidryas* egg yolks than those from the high-food rearing treatment (low-food rearing: \( 1.4 ± 1.1 \) yolks; high-food rearing: \( 0.9 ± 0.7 \) yolks), although this difference was nonsignificant (Wald \( \chi^2 = 2.83, P = 0.093 \)). After the food-deprived begging assay, when food deprivation was identical for the two treatments, tadpoles from both treatment groups ate similar amounts (low-food rearing: \( 1.5 ± 2.1 \) yolks; high-food rearing: \( 1.3 ± 1.4 \) yolks; Wald \( \chi^2 = 0.13, P = 0.722 \)).

**Laboratory assays of begging behaviour**

Tadpoles from the high- and low-food rearing treatments did not differ in the amount of time they spent begging \( (F_{1,32} = 0.03, P = 0.857 \); Fig. 2), but
tadpoles spent significantly less time begging when food-deprived ($F_{1,29} = 7.88$, $P = 0.009$; Fig. 2), and more developed tadpoles spent more time begging ($\beta \pm SE = 7.8 \pm 1.78$, $F_{1,32} = 19.42$, $P < 0.001$). The rearing treatment × hunger interaction was nonsignificant ($F_{1,29} = 2.93$, $P = 0.098$).

Tadpoles reared in the high-food treatment performed longer begging bouts than those reared in the low-food treatment ($F_{1,32} = 5.09$, $P = 0.031$; Fig. 2), as did more developed tadpoles ($\beta \pm SE = 0.61 \pm 0.11$, $F_{1,32} = 28.49$, $P < 0.001$). Tadpoles performed bouts of similar duration before and after food deprivation ($F_{1,29} = 0.85$, $P = 0.364$; Fig. 2), and there was no hunger × rearing treatment interaction ($F_{1,29} = 0.42$, $P = 0.522$; Fig. 2).

Tadpoles from both rearing treatments vibrated at similar speeds ($F_{1,32} = 0.95$, $P = 0.336$; Fig. 2c) and vibration speed was unrelated to developmental stage ($F_{1,32} = 0.42$, $P = 0.522$). Tadpoles vibrated at higher speed when satiated than when food-deprived ($F_{1,28} = 15.78$, $P < 0.001$; Fig. 2), and the rearing treatment × hunger interaction was nonsignificant ($F_{1,28} = 2.08$, $P = 0.160$; Fig. 2). There was a significant interaction between within-trial begging bout position (first, middle, last) and treatment ($F_{2,66} = 4.65$, $P = 0.013$). Vibration speed declined across the three bouts in both rearing treatments, but this decline was steeper for tadpoles from the low-food rearing treatment (Fig. 3). There was, however, no difference in the duration of first, middle and last observed begging bouts (bout number: $F_{2,68} = 1.15$, $P = 0.323$; bout number × treatment: $F_{2,66} = 0.37$, $P = 0.695$).

Maternal provisioning in the wild

Females spent 386 ± 299 s (range: 193–1916) on or in the artificial rearing cup, and 159 ± 47 (range: 26–151) s in the water, where physical contact with tadpoles was possible. The 25 tadpoles that were fed received meals of 3.4 ± 1.4 (range: 1–5) eggs. On 15 occasions, we directly observed egg laying (vs. seeing eggs after mothers departed), and these depositions occurred 77 ± 32 (range: 26–151) s after females entered the water.

As was the case in laboratory samples, developmental stage was significantly and positively associated with mean begging bout duration (Spearman’s $r = 0.531$, $n = 31$, $P = 0.002$) and the proportion of time spent begging (Spearman’s $r = 0.386$, $n = 31$, $P = 0.032$), although not with vibration speed (Spearman’s $r = 0.007$, $n = 30$, $P = 0.970$).

The number of eggs mothers provided during visits was positively, although marginally, associated with tadpole developmental stage (Wald $\chi^2 = 3.49$, $P = 0.062$; Fig. 4) and was positively and significantly associated with mean vibration speed (Wald $\chi^2 = 5.87$, $P = 0.015$; Fig. 4), but not with the proportion of time a tadpole spent begging (Wald $\chi^2 = 0.03$, $P = 0.864$) or its mean begging bout duration (Wald $\chi^2 = 1.51$, $P = 0.219$).

We observed six females visiting the nonbegging A. callidryas tadpoles that had been swapped for their own. Only one female delivered trophic eggs, while the same six females all provisioned on the first recorded visit they made to their own tadpoles (Fisher’s exact: $P = 0.015$).

Discussion

Three honest begging hypotheses – Signal of Need, Signal of Quality and Signal of Hunger – rest on different predicted relationships between offspring solicitation intensity and offspring state. We tested these predictions by independently manipulating the need/quality...
and hunger of *O. pumilio* tadpoles and assaying begging performance. The relationships between tadpole state and begging were most consistent with the Signal of Quality hypothesis, and strongly suggest that neither Signal of Need nor Signal of Hunger are good candidates to explain tadpole begging’s function and/or evolution. Contrary to the predictions of Signal of Need, tadpoles at late developmental stages (i.e. those that ‘need’ less maternal investment to reach viability: Dugas et al., 2016b) spent more time begging (see also Stynoski, 2012) and performed longer begging bouts (true in both the lab and the wild). Tadpoles experimentally reared to be in high condition (similarly in lower need: Morey & Reznick, 2000) performed longer begging bouts and demonstrated slower within-trial decline in vibration speed than did tadpoles from the low-condition treatment. Tadpole begging changed following food deprivation, but changed in a way opposite to that predicted by Signal of Hunger: tadpoles begged less and less intensely after a period of food deprivation approximating the natural interval between maternal feedings.

Fig. 2 Time *Oophaga pumilio* tadpoles from high- and low-food rearing treatments (filled circles and open triangles, respectively) spent performing vibratory begging displays during 80-second laboratory assays (a), the mean duration of begging bouts (b), and the mean speed of their vibrations, measured from 120-fps recordings (c). Estimates of central tendency are estimated marginal means from repeated-measures linear mixed models including the fixed effects of rearing treatment, hunger, the treatment x hunger interaction and developmental stage. Error bars indicate standard error. The effect of rearing treatment was significant (*P* < 0.05) only for mean begging bout duration (b), while food deprivation significantly influenced time spent begging (a) and vibration speed (c).

Fig. 3 Vibration frequency of *Oophaga pumilio* tadpoles, measured from 120-fps recordings, declined across the first, middle and last begging bout they performed during an 80-s laboratory trial. This decline was steeper in tadpoles reared in a low-food treatment (open triangles) than in those reared in a high-food treatment (filled circles). Estimated marginal means are from a repeated-measures linear mixed model including the fixed effects of treatment, hunger, developmental stage, within-trial bout position and the treatment x bout position interaction. Error bars indicate standard error.
et al. (Dugas 2016a). In captivity, overlapping broods and variation in developmental speed often present mothers with valuable tadpoles, as do captive females (Dugas et al., 2016a). In captivity, overlapping broods and variation in developmental speed often present mothers with concurrent offspring at varying stages of development (Dugas et al., 2016a,b), although the extent to which this is true in the wild is unknown. Favouritism towards high-quality offspring can be explained by the higher fitness dividends paid when trophic eggs are allocated to more developed ones (Jeon, 2008; Dugas et al., 2016b). Of particular value to testing the hypothesis that parents use offspring signals to make adaptive life-history decisions will be future studies that (i) incorporate other factors predicted to shape female choosiness and provisioning effort (e.g. brood size: Dugas et al., 2016a), (ii) document the extent to which mothers sample the signals of multiple offspring before allocating trophic eggs (Royle et al., 2002) and (iii) develop methodologies by which offspring begging effort can be manipulated.

An earlier observational study (Stynoski, 2012) revealed that wild mothers are more likely to feed when their tadpole begs, suggesting that rapid vibration functions in O. pumilio offspring–parent communication. While it is certainly plausible that the A. callidryas tadpoles we used as nonbegging stimuli differed from O. pumilio tadpoles in other ways that mothers detected, females’ lack of provisioning to those nonbegging tadpoles also suggests a communication role for rapid vibration. Without new methodologies that allow graded manipulation of tadpole vibration independent of cues like body size, it will be difficult to unequivocally demonstrate that O. pumilio mothers rely on offspring signals to make provisioning and allocation decisions. Along these lines, it remains possible that tadpole vibration evolved simply to signal tadpole presence (a similar suggestion is sometimes made for conspicuous coloration in nestling birds: Dugas, 2015). However, O. pumilio mothers often visited begging tadpoles and departed without feeding, a result inconsistent with this presence-signalling hypothesis. In O. pumilio families, any maternal ‘threshold’ for begging set above a value that all tadpoles can reach still functions in a manner consistent with Signal of Quality.

The increase in begging effort following food deprivation predicted by Signal of Hunger is nearly universal in nestling birds (Mock et al., 2011), but the logic underlying this hypothesis may not hold in other taxa. In rapidly growing nestlings (and other endotherms), the chances of catastrophic starvation probably increase with food deprivation (Grodzinski & Lotem, 2007; McCue, 2010), perhaps selecting for parental attendance to hunger. However, dire consequences of an empty gut are substantially less plausible for an O. pumilio tadpole that can survive weeks without food (Brust, 1993; Maple, 2002; McCue, 2010). Both tadpole starvation-resistance and the fact that egg-feeding mothers can leave a meal that their offspring can consume later (Perry & Roitberg, 2006) highlight the questionable utility of a hunger-based signalling system in O. pumilio and similar animals. Tadpoles food-deprived for short periods (2 h) do, however, spend remarkably little time begging (Stynoski, 2012), a pattern that might be explained outside the context of offspring–parent communication. Because O. pumilio mothers typically provision every 2 days (Maple, 2002; Stynoski, 2012), a tadpole that has eaten recently is unlikely to be fed again, and may thus be better off avoiding the energetic (Stynoski, 2012; Yoshioka et al., 2016) and predation-risk (Stynoski & Noble, 2012) costs of begging.

In short, we found that the O. pumilio tadpoles that performed a physically demanding (Wassersug & Sperry, 1977) and energetically expensive behaviour (Stynoski, 2012; Yoshioka et al., 2016) most often and most intensely were more developed, in better body condition, and had eaten recently. Although just the opposite is predicted by the popular Signal of Need hypothesis, this pattern is entirely consistent with what is known about the production of similar animal signals.
in other contexts (e.g. performance of courtship displays: Clark, 2012) and physically demanding tasks in general (McCue, 2012). The fitness benefits of favouring high-quality offspring are well-established (Temme, 1986; Haig, 1990; Davis et al., 1999; Jeon, 2008), and such favouritism is commonly observed in nature (Caro et al., 2016a). When parents benefit from favouring high-quality young and retain some control over the allocation of resources (Royle et al., 2015), the evolution of signals of quality may be common.

**Acknowledgments**

Tulane University IACUC approved all methods (R0382-R1), and MINAET (Costa Rica) issued the appropriate permit (053-2013-SINAC). This work was funded by a grant from the Louisiana Board of Regents to MBD (LEQSF-EPS(2013)-PFUND-332), and MB was supported on a grant from the National Science Foundation (Award 1146370). We are grateful to Corinne Richards-Zawacki and to the staff of La Selva Biological Station. Doug Mock, Arnon Lotem, Per Smiseth and two anonymous reviewers provided helpful comments on earlier drafts of this manuscript.

**References**


**Supporting information**

Additional Supporting Information may be found online in the supporting information tab for this article: *Video S1* Free-living *Oophaga pumilio* mothers visiting and feeding their begging tadpoles. *Video S2* Laboratory assay of *Oophaga pumilio* tadpole begging recorded at 120 fps. *Video S3* Mother *Oophaga pumilio* visiting rearing sites in which we swapped their own tadpoles for those of a locally abundant treefrog (*Agalychnis callidryas*) with non-begging larvae.

Data deposited at Dryad: doi:10.5061/dryad.sf1s3

Received 9 November 2016; accepted 7 March 2017