Both sexes pay a cost of reproduction in a frog with biparental care

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The assumption that reproduction is costly is central to life-history theory. Good evidence supporting this premise comes from studies, mostly in short-lived invertebrates, demonstrating a negative relationship between reproduction and longevity. Whether this trade-off operates broadly, for example in males and females and in short- and long-lived organisms, remains unresolved. We found a negative relationship between reproduction and days survived in captive, wild-caught, individuals of a long-lived poison frog with biparental care (*Oophaga pumilio*). The proportion of time that individuals spent paired and tadpole production rate were negatively associated with days survived in both sexes, and clutch production was negatively associated with days survived in females. These results broaden the taxonomic base upon which this tenet of life-history theory is built, empirically confirm that females of this species should be choosy when selecting mates and caring for offspring, and suggest that the costs of 'limited' male care in this species deserve re-evaluation. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, ••, ••–••.

ADDITIONAL KEYWORDS: life-history – longevity – *Oophaga pumilio* – parental care.

INTRODUCTION

A trade-off between reproduction and other fitness components is thought to be central to the evolution of life histories (Williams, 1966; Stearns, 1992). One line of evidence supporting the hypothesized costliness of reproduction demonstrates a negative relationship between reproduction and longevity (Reznick, 1985; Stearns, 1992; Flatt, 2010). Following a surge of empirical work driven by theoretical advances and syntheses, this trade-off once appeared nearly universal (Reznick, 1985; Stearns, 1992; Reznick, Nunney & Tessier, 2000). However, some more recent counterexamples have challenged this consensus, highlighting the need for more attention to

this fundamental assumption of life-history theory (Reznick *et al.*, 2000; Flatt, 2010; Wit *et al.*, 2013; Tarín *et al.*, 2014). Sampling across taxonomic and functional groups is needed to test for (near) universality of a trade-off, to identify patterns in when, where, and to what extent the trade-off occurs, and to reveal potential underlying mechanisms (Reznick, 1985). For example, a focus on small, short-lived organisms is often logistically desirable when measuring longevity. However, such species have typically evolved under high risk of extrinsic mortality, and selection for traits that lower the costs of reproduction is probably stronger when life span is long and extrinsic mortality is low (Williams, 1966; Keller & Genoud, 1997; Williams *et al.*, 2006; Wit *et al.*, 2013). In sexually reproducing organisms, the relative costs paid by females and males also have important selective consequences (Trivers, 1972). In addition to initial investment in gametes, both sexes often contribute to postzygotic care and these costs can differ between the sexes, even for the same behaviour (Santos & Nakagawa, 2012; Parker, Schwagmeyer & Mock,

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2013). Comprehensive examinations of trade-offs in males as well as females are thus critical to maximizing the explanatory power of life-history patterns (Hunt *et al*., 2001; Liker & Székely, 2005; Scharf, Peter & Martin, 2013; Cornwallis, Dean & Pizzari, 2014).

We tested the prediction that reproduction and longevity are negatively associated in a captive colony of the strawberry poison frog, *Oophaga pumilio*, a polytypic frog that is fast becoming a model system for the study of phenotypic divergence and speciation (Gehara, Summers & Brown, 2013). Despite differences between captive and wild environments, captive animals provide a good proxy for intrinsic sources of mortality in wild animals (Ricklefs, 2000) and are not subject to extrinsic confounds. In the wild, *O. pumilio* can be long-lived (≥ 5 years) and presumably because of its toxicity and aposematic colouration, suffers low mortality (Richards-Zawacki, Yeager & Bart, 2013). Successful male reproduction requires territory defence and courtship (Pröhl & Hödl, 1999), and both activities are energetically costly in anurans (Ryan, Bartholomew & Rand, 1983; Leary *et al*., 2004) and reduce longevity in other animals (Cordts & Partridge, 1996; Hunt *et al*., 2001). Males also tend clutches, periodically moistening them and perhaps consuming any eggs that begin to spoil (Weygoldt, 1980; Brust, 1993; Pröhl & Hödl, 1999; Pröhl, 2005). Any intrinsic costs of these male parental care behaviours are unclear, although similar behaviours are costly in other animals (Townsend, 1986; Gillooly & Bayliss, 1999; Visser & Lessels, 2001). Female *O. pumilio* lay reproductive clutches and transport tadpoles to small pools of water, typically in bromeliads (Weygoldt, 1980; Brust, 1993). Females then provision tadpoles with unfertilized eggs throughout larval development (Weygoldt, 1980; Brust, 1993). Egg production can be particularly expensive (Visser & Lessels, 2001; Liker & Székely, 2005; Williams, 2005), and so this provisioning behaviour presumably generates substantial intrinsic costs of reproduction for females.

This apparent imbalance in behavioural parental effort between the *O. pumilio* sexes has been interpreted as asymmetric reproductive investment (*sensu* Trivers, 1972) and evoked as a driver of strong sexual selection and thus a potential explanation for rapid phenotypic diversification in this species (Summers *et al*., 1997). Despite the popularity of this hypothesis (e.g. Richards-Zawacki & Cummings, 2011, reviewed by Gehara *et al*., 2013), its underlying assumptions have received little empirical attention (but see Pröhl & Hödl, 1999; Pröhl, 2005). To test for costly reproduction in *O. pumilio*, we followed the reproduction of wild-caught frogs held in captivity, and asked how three proxies for reproductive activity were associated

with the length of time individuals survived: (i) the proportion of time paired (vs. held without a mate), (ii) rate of clutch production, and (iii) tadpole production. If reproduction is costly, all three metrics should be negatively associated with captive longevity, although the effects might differ between the sexes. The metric 'proportion-of-time paired' captures the costs of courtship to males and those females pay for interacting with males (e.g., Magurran & Seghers, 1994). While clutch production might reflect higher costs of successful male courtship (i.e. mating), we expect this metric to be particularly costly for females because they produce the eggs (Williams, 2005). We operationally defined tadpoles as the number transported to rearing sites (Dugas, Yeager & Richards-Zawacki, 2013), so this metric captures potentially costly parental behaviours for both sexes (egg tending, tadpole transport, tadpole feeding), but is again assumed to be much more costly for females (Summers *et al*., 1997).

MATERIAL AND METHODS

In February, 2008 and August, 2009, we captured wild adult frogs from four *O. pumilio* populations in the Bocas del Toro region of Panama (see Supporting Information, Table S1), and in August 2009, we moved the frogs to Tulane University (LA, USA). Breeding pairs were housed in plastic enclosures and provided with tadpole-deposition sites (details in Supporting Information, Appendix S1 and Dugas *et al*., 2013). We monitored survival and reproduction of adults with twice-weekly censuses, noting the presence of adults, clutches, and tadpoles in rearing sites (i.e. a 'tadpole' hatched and was transported to a rearing site).

We operationally defined longevity as the number of days between the move to Tulane and death. Assuming we collected adult frogs of similar age on both dates, some frogs were ~1.5 years older than others when moved to Tulane, and so we included collection date as a fixed factor in all analyses. We consider here only adult deaths that occurred before August 2011, when we changed the frogs' diet in a way that altered reproductive success (Dugas *et al*., 2013). Before the diet change, nearly all tadpoles died prior to metamorphosis, but larval mortality typically occurred late in development (*unpublished data*), indicating that the presence of tadpoles is a good proxy for continued maternal investment via trophic eggs.

Because of non-independence of cases in which both members of a pair died ($N = 5$), we ran all analyses separately for females and males. Of the 120 wild-caught frogs used to establish the colony, 37 (17 female, 20 male) died by August, 2011. All 37 participated in pairings that produced clutches, and

22 (10 females, 12 males) in pairings that produced tadpoles. We took a two-step approach to the analysis, first asking about predictors of longevity in all individuals, and then focusing on the subset of individuals that produced tadpoles (hereafter, 'reproductively successful'). We treated reproductively successful and unsuccessful individuals as separate classes because of the myriad and unclear potential explanations for why some individuals may have not produced tadpoles, including low initial quality and mate sterility (or poor parental care); this factor should not be confused with the common experimental design of comparing reproductively active and non-active individuals (Flatt, 2010), as all study animals were paired, all produced clutches, and all were wild-caught as adults.

For each sex, we began with a linear mixed model in which longevity was entered as the dependent variable, reproductively successful (y/n), collection date, clutches per day, and proportion-of-time-paired were entered as fixed effects, and population-of-origin was included as a random effect. To examine the effect of tadpole production rate, we then restricted the analysis to individuals that produced tadpoles; we used a model identical to the one above except for the substitution of the effect 'tadpoles per day' for 'reproductively successful (y/n)'. We re-assessed the significance of terms after sequentially removing non-significant ($P > 0.10$) fixed effects.

We predicted a negative relationship between reproduction and days survived if reproduction is costly, but age-specific reproductive investment (Forslund & Pärt, 1995) could also contribute to this pattern if the individuals that died were from an older cohort and reproductive effort increases with age. We tested for evidence consistent with this explanation by first using a paired t-test to compare the size (snout-vent length) at capture of individuals that died (i.e., those included in this data set) to a matched set of individuals that did not die during the study period (we were able to match capture date, population, and sex for 22 individuals); if those individuals that died during the study period were older at its start, they should have been larger than those that survived. Size is a generally accepted proxy for age in amphibians (Duellman & Trueb, 1986), and in a population of *O. pumilio* (Richards-Zawacki *et al.*, 2013), individuals were indeed larger when recaptured 488 ± 137 days (mean ± SD) later (paired $t_{19} = -5.0$, $P < 0.001$). We then compared reproductive output of individuals from early and late collection dates; if older individuals invest more in reproduction, the earlier collected individuals should have higher reproductive rates. To compare rates, we used linear mixed models in which we entered output (clutches or tadpoles per day) as the dependent variable, collection date as a fixed effect, and population-of-origin as a random effect.

To meet the assumption of normality, we arcsine square-root transformed proportion-of-days-paired, and log_{10} transformed clutches day^{-1} and tadpoles day^{-1}; prior to transformation, we multiplied both parameters by 1000 to ensure positive values. We also explored correlations among continuous fixed effects to address potential issues stemming from multicollinearity. We used the proc MIXED command in SAS (v9.2) for all analyses, and calculated degrees-of-freedom for fixed effects with the Kenward-Roger approximation. We used two-tailed tests, but discuss marginal effects ($P < 0.10$) given small sample sizes and directional *a priori* predictions.

RESULTS

Females included in this sample produced (mean ± SD) 42.3 ± 23.5 clutches and 7.8 ± 13.5 tadpoles per 1000 days in captivity, while males were involved in pairs that produced 46.8 ± 25.2 clutches and 9.0 ± 12 tadpoles per 1000 days. Females collected on the earlier date survived 408 ± 195 days in captivity at Tulane, and females collected later survived 435 ± 147 days. Males collected on early and later dates survived 481 ± 189 days and 430 ± 138 days respectively. Reproductively successful females lived longer than females that produced no tadpoles, but days survived was negatively associated with clutches produced per day and the proportion of time a female was paired (Table 1, Fig. 1). Females from the first collection date died earlier (Table 1), and female populations-of-origin explained 92% of residual variance in survival. Clutches per day and proportion-of-time-paired were not associated ($r = 0.05$, $N = 17$, $P = 0.846$). Male captive longevity was marginally and negatively associated with proportion-of-time-paired ($P = 0.096$), but there were no significant relationships between days survived and the predictors we considered (Table 1, Fig. 1); removing non-significant fixed effects did not qualitatively change results. Male populations-of-origin explained 4% of residual variance. In males, clutch production and proportion-of-time-paired were strongly correlated ($r = 0.708$, $N = 20$, $P < 0.001$) raising concerns about multicollinearity. We present the model containing only proportion-of-days-paired (Table 1, Fig. 1) because it provided better overall model fit (in the alternative, clutch production per day: $F_{1,12.5} = 0.71$, $P = 0.415$).

In the subset of females that produced tadpoles, there was a marginal ($F_{1,5} = 4.8$, $P = 0.080$) negative relationship between tadpole production per day and days survived (Fig. 2A), but neither the effects of clutch production ($F_{1,5} = 3.2$, $P = 0.135$), proportion-of-

Table 1. Results of linear mixed models examining predictors of wild-caught *O. pumilio* longevity (days) when held in captivity. β and standard error (SE) values for reproductively successful (y/n) reflect the effect of 'no' and for collection date, reflect the effect of 'early'. Clutches per day was Log_{10} ($n*1000$) transformed, and proportion-of-time-paired was $\arcsin \sqrt{n}$ transformed prior to analysis

	F	d.f.	P	β	SE
Females					
Intercept				2368.7	427.7
Reproductively successful (y/n)	9.5	1,9.9	0.012	−190.6	61.8
Clutches per day	5.4	1,10.2	0.042	−266.6	114.8
Proportion-of-time paired	26.9	1,10.4	< 0.001	−969.4	186.8
Collection date	13.7	1,10.6	0.004	−245.5	66.5
Males					
Intercept				972.5	285.2
Reproductively successful (y/n)	2.16	1,16	0.161	−142.5	97.1
Proportion-of-time paired	3.14	1,15.6	0.096	−384.8	217.2
Collection date	0.34	1,7.47	0.576	58.7	100.5

time-paired ($F_{1,5} = 2.6$, $P = 0.168$) nor collection date ($F_{1,5} = 1.8$, $P = 0.236$) were significant, and removing non-significant effects did not qualitatively change results. For males, no predictors were significant in the full model (Table S2), but sequential removal of non-significant terms produced a model containing only tadpole production per day, which was negatively associated with days survived ($r^2 = 0.49$, $F_{1,10} = 9.6$, $P = 0.011$; Fig. 2B). In this subset of observations, population-of-origin explained < 1% of residual variance in both sexes, and no continuous fixed effects were significantly correlated (all r: −0.282–0.475, $N = 12$ or 10, all $P > 0.118$).

Individuals that died during the study period were no larger than those that did not (paired $t_{21} = -0.8$, $P = 0.408$), suggesting that they were not drawn from a different age cohort. Reproductive output was no higher in individuals captured at earlier collection dates, as we would expect if investment increased with age: Neither clutch nor tadpole production differed between females collected early and late (clutches per day: $F_{1,14.9} = 0.3$, $P = 0.59$; tadpoles per day: $F_{1,7.6} = 0.02$, $P = 0.88$). For males, clutch rate did not differ between collection dates ($F_{1,13.9} = 0.7$, $P = 0.42$), and while there was a marginal difference in tadpole production between frogs collected early rather than late ($F_{1,10} = 3.36$, $P = 0.097$), it was males from the second collection that produced more tadpoles per day.

DISCUSSION

Patterns in a captive colony of *O. pumilio* were consistent with the hypothesis that reproduction comes at the cost of reduced longevity. Because we studied wild-caught frogs with unknown reproductive histo-

ries, these results must be treated with some caution, and future experimental approaches can build on predictions made from the results of this correlative analysis. For both sexes, the proportion of their captive lives spent interacting with a mate was negatively associated with days survived, as was the rate of tadpole production, while the rate of clutch production was associated with days survived only for females. Females that produced ≥ 1 tadpole lived longer than those that produced none, although this pattern seems unlikely to be a result of some intrinsic benefit of tadpole production. These were all wild-caught females that produced clutches in the presence of a male (i.e., were not unmated), and differences in initial quality or mate fertility between groups seem the safest interpretation of this pattern (Forslund & Pärt, 1995). The importance of intrinsic costs of reproduction to life-history evolution depends, among other things, on the relative importance of extrinsic and intrinsic sources of mortality as selective pressures (Williams, 1966; Keller & Genoud, 1997; Williams *et al.*, 2006). *O. pumilio* are long lived and adult survivorship can be high (Richards-Zawacki *et al.*, 2013), so the patterns revealed in this study could be important in nature.

The negative relationship between proportion of time paired and days survived could reflect, for both sexes, metabolic and endocrine costs associated with entering a reproductive state. Alternatively or additionally, males might incur direct costs of courtship displays (Ryan *et al.*, 1983; Cordts & Partridge, 1996; Hunt *et al.*, 2001; Leary *et al.*, 2004). It is not obvious how being courted would be costly for captive females, although in other animals female foraging is reduced by male harassment (Magurran & Seghers, 1994). However, such costs could be a laboratory artefact, as
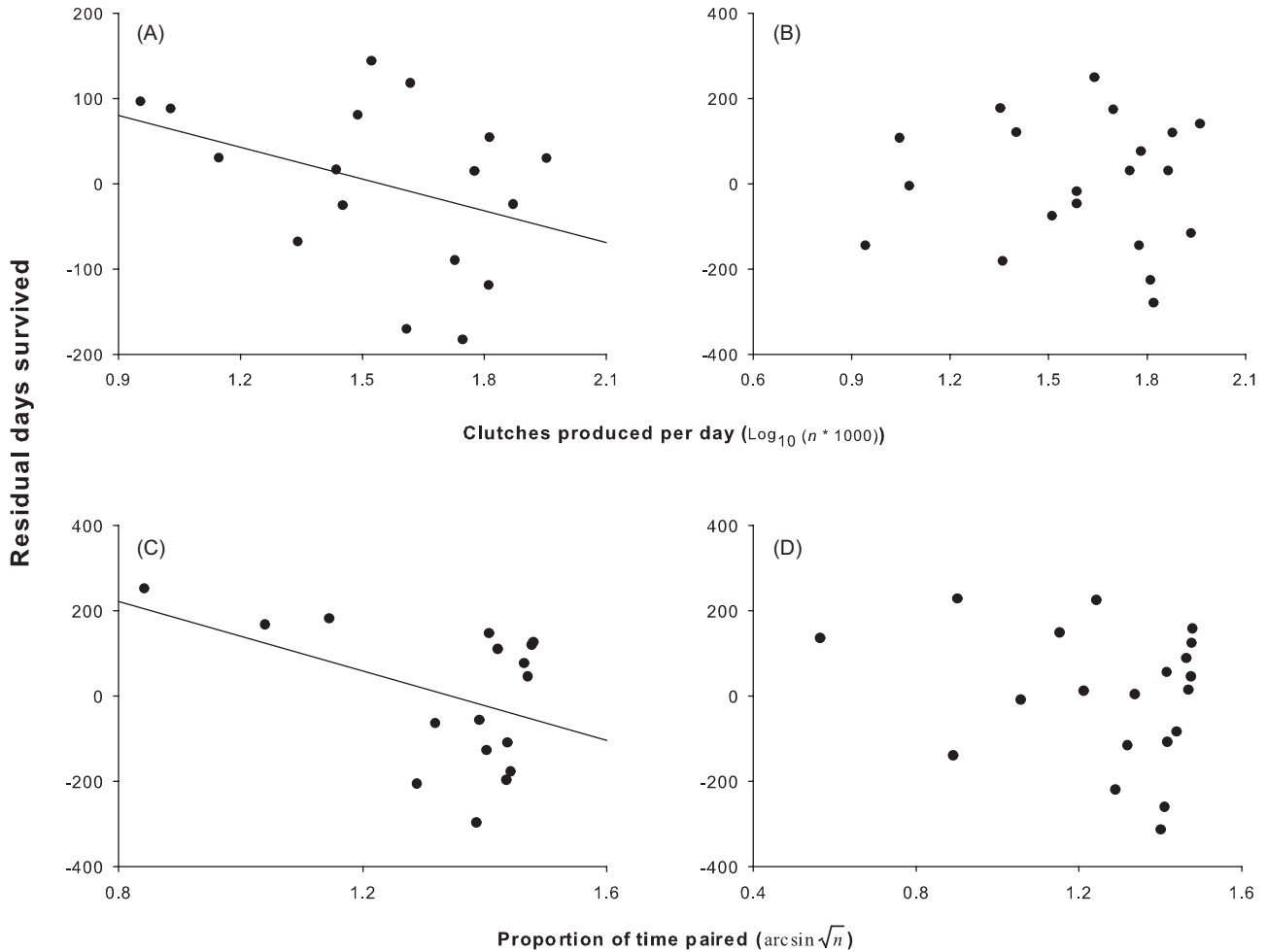
Figure 1. The relationships between days survived by *O. pumilio* in a captive breeding colony and clutch production per day (females in A, males in B) or proportion of their captive lives spent paired with an individual of the opposite sex (females in C, males in D). Days survived is presented as residuals from a model that contained both these metrics as well as the fixed effects of collection date and reproductively successful (y/n) and the random effect of population-of-origin. Regression lines are presented for significant ($P < 0.05$) relationships.

wild females spend a large proportion of their time away from male territories, and wild males divide their interest among multiple females (Pröhl & Hödl, 1999).

The costs of egg production, paid by females at the clutch and tadpole stage, probably explain the negative relationships between days survived and clutch and tadpole production in *O. pumilio* females (Visser & Lessels, 2001; Williams, 2005). This negative relationship between female reproduction and days survived lends important empirical support to the assumption that females pay a cost for egg production and should be choosy when selecting mates and feeding offspring (Trivers, 1972; Summers *et al.*, 1997; see also Pröhl & Hödl, 1999). Male care is concentrated on developing eggs (Weygoldt, 1980; Pröhl & Hödl, 1999), so the best explanation for the negative

relationship between tadpole production and days survived in males is probably that successfully caring for clutches is costly. Paternal care is not uncommon in frogs with terrestrial eggs and can be energetically costly (e.g., Townsend, 1986; Vockenhuber, Hödl & Amezquita, 2009), and limited male care can drive female mate choice and mate guarding (Wells, 1978; Summers, 1989, 1992). The intrinsic mechanisms by which care might be costly to *O. pumilio* fathers are unclear, but fungal growth on clutches is common in the wild (Pröhl, 2005) and in our colony, and it seems plausible that consuming spoiled eggs (Brust, 1993) could occupy valuable gut space or present males with costly immune challenges.

The relationship between relative parental investment and sexual selection (Trivers, 1972) is well established, although tallying of the costs of such
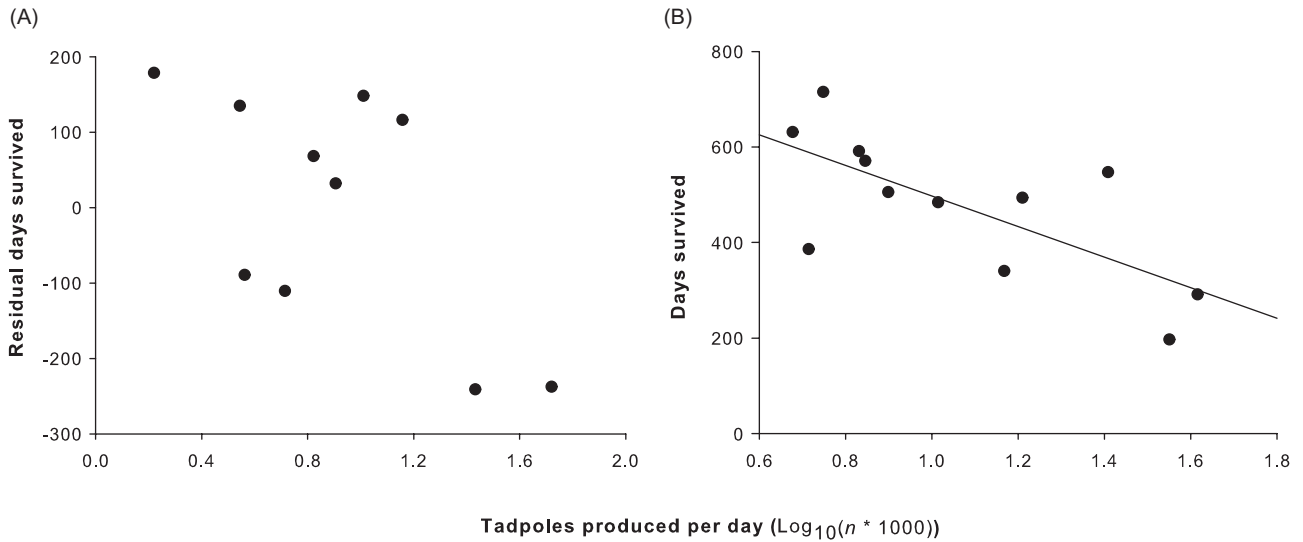
Figure 2. The relationships between tadpole production per day and female (A) and male (B) *O. pumilio* days survived in captivity. Female days survived is presented as residuals from a model that also contained the fixed effects of collection date, clutch production per day and proportion-of-time-paired, while the relationship for males is a linear regression (the best model). The regression line indicates a significant ($P < 0.05$) relationship.

investment is far from simple, especially when investment extends past the zygote stage (Monaghan & Nager, 1997; Nilsson, 2002; Liker & Székely, 2005; Selman *et al*., 2012). Moreover, the costs (and benefits) of care can be sex-specific even for the same behaviour (Santos & Nakagawa, 2012; Parker *et al*., 2013), highlighting the limited utility of behavioural proxies for investment. Determining the sum of extrinsic and intrinsic costs paid by each sex in *O. pumilio* will require careful experimental and observational work (e.g. Pröhl & Hödl, 1999; Pröhl, 2005), and is unlikely to be static across contexts. For example, the operational sex ratio (OSR), and thus intensity of sexual selection, is sensitive to the rate of clutch failure in *O. pumilio* populations (Pröhl, 2005). As clutch failure increases, males spend proportionately more of their time in parental care activities, leading to a less male-biased OSR (Pröhl, 2005); intrinsic costs of clutch tending (this paper) are likely to exaggerate such reductions in the intensity of sexual selection. The extent to which male care is itself under selection should depend on the variation in the quality of care males provide, in other words whether male care can reduce clutch mortality (Summers, 1992). Our findings indicate that better quantification of the extent, costs, and benefits of male egg care in *O. pumilio* is warranted and may lend new insights into the role sexual selection could have played in the notable phenotypic diversification of this polytypic frog (Summers *et al*., 1997; Pröhl, 2005).

This study supports the hypothesis that reproduction carries a longevity cost, and that this trade-off could explain broad patterns of life-history variation. Beyond simply asking whether this trade-off occurs, future work can address among-species or among-population variation in its presence and/or extent, using comparative work to identify drivers of the often complex relationship between reproduction and longevity (Williams, 1966; Williams *et al*., 2006). Isolated populations of this and similar species vary extensively in colouration, and likely experience different predation risk (Rudh, Breed & Qvarnström, 2013; Willink *et al*., 2014). Among-population comparisons in these frogs may, then, provide an excellent opportunity to examine the role of extrinsic mortality in mediating the evolution of life-history trade-offs (Williams, 1966; Williams *et al*., 2006). Identifying the mechanistic links mediating a reproduction–longevity relationship promises further refinement of its explanatory and predictive power (Harshman & Zera, 2007). Substantial recent attention has been paid to oxidative stress caused by the high metabolic rate associated with reproduction (Nilsson, 2002; Selman *et al*., 2012; Metcalfe & Monaghan, 2013). Because poison frogs vary extensively in reproductive behaviour and metabolic rate (Santos & Cannatella, 2011), they may prove an excellent system in which to test for such proximate mechanisms and to explore their consequences for life-history evolution.

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REFERENCES

Brust DG. 1993. Maternal care by *Dendrobates pumilio*: a frog that feeds its young. *Journal of Herpetology* **27:** 96–98.

Cordts R, Partridge L. 1996. Courtship reduces longevity of male *Drosophila melanogaster*. *Animal Behaviour* **52:** 270–278.

Cornwallis CK, Dean R, Pizzari T. 2014. Sex-specific patterns of aging in sexual ornaments and gametes. *American Naturalist* **184:** E66–E88.

Duellman WE, Trueb L. 1986. *Biology of Amphibians*. Baltimore: Johns Hopkins University Press.

Dugas MB, Yeager J, Richards-Zawacki CL. 2013. Carotenoid supplementation enhances reproductive success in the captive strawberry poison frogs (*Oophaga pumilio*). *Zoo Biology* **32:** 655–658.

Flatt T. 2010. Survival costs of reproduction in *Drosophila*. *Experimental Gerontology* **46:** 369–375.

Forslund P, Pärt T. 1995. Age and reproduction in birds – hypotheses and tests. *Trends in Ecology and Evolution* **10:** 374–378.

Gehara M, Summers K, Brown JL. 2013. Population expansion, isolation and selection: novel insights on the evolution of color diversity in the strawberry poison frog. *Evolutionary Ecology* **27:** 797–824.

Gillooly JF, Bayliss JR. 1999. Reproductive success and the energetic cost of parental care in male smallmouth bass. *Journal of Fish Biology* **54:** 573–584.

Harshman LG, Zera AJ. 2007. The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution* **22:** 80–86.

Hunt J, Brooks R, Jennions MD, Smith MJ, Bentsen CL, Bussière LF. 2001. High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432:** 1024–1027.

Keller L, Genoud M. 1997. Extraordinary lifespans in ants: tests of evolutionary theories of ageing. *Nature* **39:** 958–960.

Leary CJ, Jessop TS, Garcia AM, Knapp R. 2004. Steroid hormone profiles and relative body condition of calling and satellite toads: implications for proximate regulation of behavior in anurans. *Behavioral Ecology* **15:** 313–320.

Liker A, Székely T. 2005. Mortality costs of sexual selection and parental care in natural populations of birds. *Evolution* **59:** 890–897.

Magurran AE, Seghers BH. 1994. A cost of sexual harassment in the guppy, *Poecilia reticulata*. *Proceedings of the Royal Society of London B* **258:** 89–92.

Metcalfe NB, Monaghan P. 2013. Does reproduction cause oxidative stress? An open question. *Trends in Ecology and Evolution* **28:** 347–350.

Monaghan P, Nager RG. 1997. Why don't birds lay more eggs? *Trends in Ecology and Evolution* **12:** 270–274.

Nilsson J. 2002. Metabolic consequences of hard work. *Proceedings of the Royal Society of London B* **269:** 1735–1739.

Parker GA, Schwagmeyer PL, Mock DW. 2013. The asymmetric incubation game: a prospective model and a house sparrow investigation. *Animal Behaviour* **93:** 37–47.

Pröhl H. 2005. Clutch loss affects the operational sex ratio in the strawberry poison frog, *Dendrobates pumilio*. *Behavioral Ecology and Sociobiology* **58:** 310–315.

Pröhl H, Hödl W. 1999. Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio*. *Behavioral Ecology and Sociobiology* **46:** 215–220.

Reznick D. 1985. Costs of reproduction: an evaluation of empirical evidence. *Oikos* **44:** 257–267.

Reznick D, Nunney L, Tessier A. 2000. Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology and Evolution* **15:** 421–425.

Richards-Zawacki CL, Cummings ME. 2011. Intraspecific reproductive character displacement in a polymorphic poison dart frog, *Dendrobates pumilio*. *Evolution* **65:** 259–267.

Richards-Zawacki CL, Yeager J, Bart HPS. 2013. No evidence for differential survival or predation between sympatric color morphs of an aposematic poison frog. *Evolutionary Ecology* **27:** 783–795.

Ricklefs RE. 2000. Intrinsic aging-related mortality in birds. *Journal of Avian Biology* **31:** 103–111.

Rudh A, Breed MF, Qvarnström A. 2013. Does aggression and explorative behaviour decrease with lost warning coloration? *Biological Journal of the Linnean Society* **108:** 116–126.

Ryan MJ, Bartholomew GA, Rand AS. 1983. Energetics of reproduction in a neotropical frog, *Physalaemus pustulosus*. *Ecology* **64:** 1456–1462.

Santos ESA, Nakagawa S. 2012. The costs of parental care: a meta-analysis of the trade-off between parental effort and survival in birds. *Journal of Evolutionary Biology* **25:** 1911–1917.

Santos JC, Cannatella DC. 2011. Phenotypic integration emerges from aposematism and scale in poison frogs. *Proceedings of the National Academy of Sciences of the United States of America* **108:** 6175–6180.

Scharf I, Peter F, Martin OY. 2013. Reproductive trade-offs and direct costs for males in arthropods. *Evolutionary Biology* **40:** 169–184.

Selman C, Blount JD, Nussey DH, Speakman JR. 2012. Oxidative damage, ageing, and life-history evolution: where now? *Trends in Ecology and Evolution* **27:** 570–577.

Stearns SC. 1992. *The evolution of life histories*. London: Oxford University Press.

Summers K. 1989. Sexual selection and intra-female competition in the green poison-dart frog, *Dendrobates auratus*. *Animal Behaviour* **37:** 797–805.

Summers K. 1992. Mating strategies in two species of dart-poison frogs: a comparative study. *Animal Behaviour* **43:** 907–919.

Summers K, Bermingham E, Weigt L, McCafferty S, Dahlstrom L. 1997. Phenotypic and genetic divergence in three species of dart-poison frogs with contrasting parental behavior. *Journal of Heredity* **88:** 8–13.

Tarín JJ, Gómez-Piquer V, García-Palomares S, García-Pérez MA, Cano A. 2014. Absence of long-term effects of reproduction on longevity in the mouse model. *Reproductive Biology and Endocrinology* **12:** 84.

Townsend DS. 1986. The costs of male parental care and its evolution in a neotropical frog. *Behavioral Ecology and Sociobiology* **19:** 187–195.

Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B, ed. *Sexual selection and the descent of man, 1871–1971*. Aldine: Chicago, 136–177.

Visser ME, Lessels CM. 2001. The costs of egg production and incubation in great tits (*Parus major*). *Proceedings of the Royal Society London B* **268:** 1271–1277.

Vockenhuber EA, Hödl W, Amezquita A. 2009. Glassy fathers do matter: egg attendance enhances embryonic survivorship in the glass frog *Hyalinobatrachium valerioi*. *Journal of Herpetology* **43:** 340–344.

Wells KD. 1978. Courtship and parental behavior in a Panamanian poison-arrow frog (*Dendrobates auratus*). *Herpetologica* **34:** 148–155.

Weygoldt P. 1980. Complex brood care and reproductive behavior in captive poison-arrow frogs, *Dendrobates pumilio* O. Schmidt. *Behavioral Ecology and Sociobiology* **7:** 329–332.

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. *American Naturalist* **100:** 687–690.

Williams PD, Day T, Fletcher Q, Rowe L. 2006. The shaping of senescence in the wild. *Trends in Ecology and Evolution* **21:** 458–463.

Williams TD. 2005. Mechanisms underlying the costs of egg production. *Bioscience* **55:** 9–48.

Willink B, García-Rodriguez A, Bolaños F, Prohl H. 2014. The interplay between multiple predators and prey colour divergence. *Biological Journal of the Linnean Society* **113:** 580–589.

Wit J, Sarup P, Lupsa N, Malte H, Frydenberg J, Loeschcke V. 2013. Longevity for free? Increased reproduction with limited trade-offs in *Drosophila melanogaster* selected for increased life span. *Experimental Gerontology* **48:** 349–357.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Number (*N*) of wild-caught *O. pumilio* from four populations in Bocas del Toro, Panama, broken down by collection date (February 2008 or August 2009), that were used to establish a breeding colony at Tulane University (New Orleans, LA, USA), and the number of individuals that died of natural causes between initial establishment (15 August 2009) and 15 August 2011.

Table S2. Full results of a linear mixed model examining predictors of the longevity (days) of male *O. pumilio* that produced ≥1 tadpole when held in captivity. β and standard error (SE) values for collection date reflect the effect of 'early'. Tadpoles per day and clutches per day were Log_{10} ($n*1000$) transformed, and proportion-of-time-paired was $\arcsin\sqrt{n}$ transformed prior to analysis.

Appendix S1. Details of animal housing and care.