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Oxygen Limitation at the Larval Stage and the Evolution of Maternal Investment per Offspring in Aquatic Environments

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abstract: Oxygen limitation and surface area to volume relationships of the egg were long thought to constrain egg size in aquatic environments, but more recent evidence indicates that egg size per se does not influence oxygen availability to embryos. Here, we suggest that investment per offspring is nevertheless constrained in aquatic anamniotes by virtue of oxygen transport in free-living larvae. Drawing on the well-supported assumption that oxygen limitation is relatively pronounced in aquatic versus terrestrial environments and that oxygen limitation is particularly severe in warm aquatic environments, we employ comparative methods in the Amphibia to investigate this problem. Across hundreds of species and two major amphibian clades, the slope of species mean egg diameter over habitat temperature is negative for species with aquatic larvae but is positive or neutral for species featuring terrestrial eggs and no larvae. Yet across species with aquatic larvae, the negative slope of egg diameter over temperature is similar whether eggs are laid terrestrially or aquatically, consistent with an oxygen constraint arising at the larval stage. Finally, egg size declines more strongly with temperature for species that cannot breathe aerially before metamorphosis compared with those that can. Our results suggest that oxygen transport in larvae (not eggs) constrains investment per offspring. This study further extends the generality of temperature-dependent oxygen limitation as a mechanism driving the temperature-size rule in aquatic systems.

Keywords: temperature-size rule, TSR, maternal effects, egg size, surface area to volume, body size.

Introduction

Maternal effects on body size reflect the extent to which mothers provision offspring, and these effects have a genetic basis (Willham 1963, 1972; Hadfield 2012). Theory suggests that the optimal level of maternal investment per offspring should balance the fitness gains accrued from increasing offspring size against the losses resulting from reduced fecundity (Lack 1947; Smith and Fretwell 1974). Given that maternal provisioning is heritable (Wilson et al. 2005) and selection on provisioning is pervasive (Rollinson and Rowe 2015; Thomson et al. 2017), this trait will vary among environments by virtue of differences in the offspring size-fitness function and/or changes in the trade-off function that maps investment per offspring to parental fecundity (Pianka 1976; Rollinson and Hutchings 2013*a*, 2013*b*). Although many selective agents may influence the evolution of offspring investment (Brockelman 1975; McGinley et al. 1987; Rollinson and Rowe 2016), oxygen availability has been described as a major factor influencing the evolution of offspring size in aquatic environments.

Both classical and contemporary literature suggests that when investment per offspring is packaged in aquatic eggs (i.e., many fish, amphibians, and invertebrates), then the evolution of increased investment is constrained by oxygen availability (Krogh 1959; Martin et al. 2017). Dissolved oxygen at the egg surface is the only oxygen available to embryos, and oxygen must diffuse through the egg membrane before becoming available for respiration (Daykin 1965). As the size of an egg increases, its volume expands more quickly that its surface area, and metabolic demand for oxygen will outstrip oxygen supply more readily for large embryos, provided that two assumptions are satisfied (Krogh 1959): metabolic oxygen demand is proportional to egg volume, and oxygen supply is proportional to egg surface area. This problem is potentially compounded by the relatively low oxygen availability in aquatic systems (Verberk et al. 2011) and by the fact that eggs are immobile, where respiration can result in a hypoxic boundary layer that forms around the egg and further impedes oxygen supply (Daykin 1965).

The hypothesis that egg size is constrained in aquatic systems dominated the theoretical and empirical literature for decades, especially for fishes (Sargent et al. 1987; Flem-

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ing and Gross 1990; Quinn et al. 1995; Hendry et al. 2001; Martin et al. 2017) but also for the Amphibia (Bradford 1990; Seymour and Bradford 1995; Seymour 1999). More recently (and contrary to the historical paradigm), it was recognized that egg surface area expands relatively quickly relative to metabolic oxygen demand of the embryo (Einum et al. 2002; Rombough 2007). Rapid expansion of surface area for gas exchange relative to metabolic demand implies that metabolic oxygen demand is not proportional to egg volume, which is likely because a large portion of the embryo comprises yolk, which is essentially metabolically inactive. Further, the yolk often occupies the centermost position in the egg, and the embryonic tissue is often positioned close to the surface of the egg capsule, facilitating diffusive oxygen uptake and minimizing differences in oxygen diffusion distance related to egg size (Rombough 1989). The fact that oxygen diffusion distance remains relatively constant whereas surface area per unit oxygen uptake increases with egg size indicates that large eggs have a greater than expected oxygen delivery capacity (Einum et al. 2002; Rombough 2007).

The prevailing view today is that oxygen availability does not constrain egg size per se (Rombough 1989, 2007; Einum et al. 2002; Hendry and Day 2003; Braga Goncalves et al. 2015; but see Woods 1999; Martin et al. 2017). However, this view should not be extended to infer that investment per offspring is not constrained by oxygen limitation, for two reasons. First, after hatching, increased activity results in increased metabolic oxygen consumption, yet respiratory features of larvae are not always well developed at early life stages (Burggren and West 1982; Burggren and Pinder 1991). This can pose a respiratory problem for large larvae because metabolic rate is elevated but oxygen acquisition depends entirely on diffusion. In fishes, for instance, the oxygen partial pressure at which routine metabolic rate becomes dependent on the environment (the critical oxygen level) is higher for large versus small salmon larvae immediately following egg hatching (Rombough 2007). This relationship between initial egg size and oxygen limitation arises because metabolic oxygen consumption increases throughout ontogeny (Rombough 1989), and after hatching, larvae must absorb oxygen cutaneously before the development of external gills, such that larger larvae likely have a small surface area for oxygen exchange relative to metabolic consumption. Therefore, in some fishes at least, a large investment per offspring comes at the cost of reduced capacity for oxygen delivery to larval offspring (Rombough 2007). A similar argument can be made for amphibians, given that oxygen consumption increases with larval size (Feder 1982; Gatten et al. 1992; Ultsch et al. 1999) and that a substantial portion of gas exchange in posthatching larvae occurs cutaneously, when gills and lungs are underdeveloped (Burggren and West 1982).

There is a second, related reason why oxygen limitation may constrain investment per offspring in aquatic systems, the premise of which falls again on increases in metabolic rate across ontogeny. Diffusion may be adequate to supply embryos and very small organisms with oxygen, but it may be inadequate for larger organisms (Burggren and Pinder 1991) because metabolic oxygen consumption expands rapidly with increases in the mass of metabolically active tissues (Gatten et al. 1992). In fact, the ontogenetic timing of gill and lung development in some fish and amphibians seems to correspond with size-related declines in the efficacy of oxygen diffusion as a primary means for gas exchange, implying that cutaneous oxygen uptake alone cannot sustain lower vertebrates as they increase in size (Burggren and Pinder 1991). But even after larvae develop specialized respiratory tissues (such as gills), ventilation of these tissues requires oxygen, and ventilation is energetically expensive for small aquatic organisms where viscous forces dominate (Moran and Woods 2012; Verberk and Atkinson 2013). When aquatic environments become hypoxic, then increases in the rate of ventilation cannot compensate indefinitely for large larval size, and smaller size with lower metabolic oxygen demand will be favored (Atkinson et al. 2006).

Aquatic environments that are warm tend to have very low levels of dissolved oxygen (Verberk et al. 2011), and ectotherms at many different life stages may therefore have difficulty regulating oxygen supply and demand in these environments (Verberk et al. 2011; Verberk and Atkinson 2013). The problem is that oxygen solubility in water is highly temperature dependent, whereas the oxygen diffusion coefficient is not. Consequently, increases in temperature will both increase metabolic oxygen demand and decrease oxygen availability (Woods 1999); this is particularly relevant under wild conditions where reduced flow rate limits dissipation of the boundary layer, a layer of oxygen-poor water that envelops a respiring organism at rest (Ultsch et al. 1999; Martin et al. 2017). Indeed, plastic responses of adult body size to temperature tend to be negative (Atkinson 1994), especially in aquatic environments (Forster et al. 2012; Horne et al. 2015), a pattern that likely reflects an integrated acclimation response to both increase oxygen supply and reduce demand (Atkinson et al. 2006).

It remains an open question whether temperature-dependent oxygen limitation can help explain the evolution of investment per offspring in aquatic environments. On the one hand, reviews by Atkinson (1994) and Atkinson et al. (2001) show that females of many species plastically reduce both adult and offspring size in warm environments, both terrestrial and aquatic, suggesting that temperature-dependent oxygen limitation drives plasticity of investment per both offspring and adult size. On the other hand, in a review of metazoan species, Forster et al. (2012) found no evidence that aquatic species have steeper temperature–offspring size reaction norms than terrestrial species (see also Forster et al. 2011), although reaction norm slopes were indeed stronger in aquatic versus terrestrial environments for adult size. Notably, no study other than that of Forster et al. (2012) has attempted to test whether temperature-dependent oxygen limitation may affect the evolution of investment per offspring very broadly, and many published associations between offspring size and temperature make no attempt to clarify how temperature and oxygen might interact to affect offspring size (e.g., Bradford 1990; Fleming and Gross 1990; Braun et al. 2013; Feiner et al. 2016; Thatje and Hall 2016).

In sum, a weight of evidence suggests that egg size per se is not limited by geometric and metabolic constraints on oxygen delivery in aquatic environments, but it is unclear whether maternal effects on larval body size resulting from investment per offspring are constrained by oxygen limitation. In this study, we leverage the diversity of development modes within the Amphibia to perform a phylogenetically explicit investigation of how investment per offspring evolves in response to oxygen limitation at the larval stage. We test four predictions; first, that investment per offspring is negatively related to temperature in species with aquatic larvae; second, that the negative relationship between investment per offspring and temperature is similar for species with aquatic eggs and aquatic larvae versus species with terrestrial eggs and aquatic larvae; third, that investment per offspring is more negatively associated with temperature in species with aquatic larvae than in terrestrial-breeding species without larvae; and fourth, that egg size of species unable to obtain pulmonary oxygen at the larval stage exhibits a stronger negative association with environmental temperature than those that can breathe aerially during the larval stage. Finally, we also perform phylogenetic path analysis (von Hardenberg and Gonzalez-Voyer 2013) to estimate whether the evolution of investment per offspring is associated with temperature or whether changes in investment per offspring are simply associated with correlated changes in clutch size or adult size that are themselves associated with temperature (Angilletta et al. 2006). Overall, our data are consistent with the hypothesis that investment per offspring evolves in response to oxygen limitation, and we provide evidence that the oxygen constraint arises during the larval stage rather than the egg stage.

Methods

Amphibians as a Model System

Amphibian diversity rivals that of other terrestrial vertebrate groups, comprising nearly 7,000 species in the order Anura (frogs and toads), about 700 species in Caudata (salamanders and newts), and about 200 species in the order Gymnophiona (caecilians; AmphibiaWeb 2016). Importantly, amphibian lifestyle and mode of respiration vary remarkably among clades and across ontogeny (Wells 2010), providing an opportunity to compare how temperature and oxygen availability may affect the evolution of body size across life stages. Amphibians are therefore well suited for the comparative approach that underlies broad exploration of temperature, oxygen availability, and the evolution of size traits.

Life-History Data

Data on egg diameter (mm), snout to vent length (SVL; mm), development mode (exotrophic larvae or direct development), egg placement (aquatic, terrestrial, foam nests, or soma [on the parent's body in, e.g., a brood pouch]), ability of larvae to respire aerially before metamorphosis (i.e., yes or no), and clutch size of frogs was collected from the literature. Species with endotrophic larvae (i.e., free-living larvae that do not feed) are relatively uncommon among the Anura, and they were not included in our analyses because obtaining information on their developmental environment was difficult (e.g., aquatic vs. terrestrially within the jelly matrix vs. terrestrially in leaf litter). We relied on a previous compilation by Gomez-Mestre et al. (2012) and on online databases, such as AmphibiaWeb (2016) and Encyclopedia of Life (Parr et al. 2014); we also used species-specific literature searches. For online databases and primary literature, it was common for a range of values to be reported, in which case we used the midpoint of the range. Data on egg diameter and SVL from previous compilations were generally point estimates, and these point estimates were used in our analysis. For all estimates, however, when several averages or medians from different populations or academic sources were obtained, we used the median of these values. In total, we obtained estimates of egg diameter, development mode, and adult SVL for 558 species, representing 80% of known families (44 of 55) and 8.5% of known species (558 of 6,584 species; AmphibiaWeb 2016).

We compiled a database for salamander life-history traits. Egg diameter, SVL, development mode, egg placement (aquatic or terrestrial), and clutch size were compiled from literature searches, books, AmphibiaWeb, and Encyclopedia of Life. A single species-wide estimate of egg diameter and SVL was obtained, as for the Anura (above). In total, we obtained egg diameter and adult SVL from 159 species comprising 10 taxonomic families, representing 100% of described families (10 of 10 families) and 23.4% of known species (159 of 680 species; AmphibiaWeb 2016). All data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h6qp4 (Rollinson and Rowe 2018).

Environmental and Geographic Data

Range size (km^2) and air temperature data were collected by Sodhi et al. (2008), where range size data were extracted from the Global Amphibian Assessment (International Union for Conservation of Nature 2006), and temperature data are based on average climatic data, interpolated from weather stations located on a 30-arcsecond resolution grid within the geographic range of each species (Hijmans et al. 2005). Mean maximum monthly temperature (hereafter maximum temperature) represents maximum daily temperature (interpolated from weather stations within each species' range) averaged within each month and then across all months in the year (for further details, see Hijmans et al. 2005; Sodhi et al. 2008). We focus on this metric of temperature in this study because, given the available temperature data, it is likely to be the better indicator of the extent to which temperature-dependent oxygen limitation occurs. Amphibian larvae overwhelmingly inhabit shallow bodies of water because many species breed in temporary sites such as vernal pools, and the deeper areas of streams and lakes are more likely to host predators such as fish (Wells 2010). Shallow water bodies are subject to strong diurnal variation in temperature, and provided that movement to deeper and cooler water is either impossible (e.g., vernal pools) or restricted (e.g., predation risk), then amphibian larvae have limited thermoregulatory scope and must be able to tolerate daily temperature maximums. For this reason, maximum temperature should be better suited for exploring whether temperature-dependent oxygen limitation occurs, because mean temperature is strongly influenced by daily minimum temperature, and minimum temperature should have less bearing on temperature-dependent oxygen limitation. For transparency, in supplementary analyses we present all results using mean temperature instead of maximum temperature, and we note where discrepancies occur between these two different temperature metrics.

In this study, we use temperatures averaged across the entire year (i.e., maximum temperature) as our estimate of environmental temperature. In our analyses, we therefore assume that variation in maximum temperature captures variation in biologically relevant temperatures experienced during the larval period, although in reality the timing and duration of the larval period will vary by species and geographic location. We performed a simple simulation that suggests that, across a large geographic gradient, there should exist a positive but imperfect correlation between maximum temperature, as defined above, and the mean maximum temperature of randomly selected intervals within the year, even when temperature intervals are allowed to vary in starting time and duration across simulation runs (table A1; tables A1–A16 are available online). We therefore suggest that using maximum temperature as a proxy for biologically relevant temperatures is reasonable, but we underline that our temperature metric is imperfect, and our results must be interpreted with this in mind.

Geographic Range Size and Spatial Scale of Analysis

If temperature drives life-history evolution, as is hypothesized, then there may be local adaptation to temperature within the geographic range of the species, which will generate geographic variation in life-history traits within species. Yet each datum in this study represents a single species-wide estimate of a given life-history trait, and in most cases it is not known where within a species' geographic range the life-history data come from. Mean temperature averaged across a species' range is therefore not necessarily representative of the mean temperature experienced by the population(s) from which a given datum was obtained. To help abate this problem, we assume that the amount of climatic variation experienced across a species' range is proportional to its geographic range size. By extension, we assume that our life-history data is well matched to climatic data for species with small range sizes but that climatic data and life-history data are on average less well matched for species with large range sizes.

Our focus is therefore on species with small geographic range sizes. To choose an appropriate spatial scale of analysis, we define small range sizes as 12,100 km^2 or less, which is the geographic area used by both Olalla-Tárraga and Rodríguez (2007) and Adams and Church (2011) in analyses of associations between amphibians and temperature. Where possible, we first performed our main analyses using data from only species with a range size of up to 12,100 km^2. These analyses provide strong support to our main predictions (see "Results"). However, excluding species with range sizes >12,100 km^2 generally resulted in poor taxonomic representation (table 1). To obtain a better taxonomic representation, we expanded the list of eligible species to those with a range size of up to 50,000 km^2 (table 1). Here, we focus on analyses of species with range sizes of up to 50,000 km^2 because these results are qualitatively the same as those obtained at a scale of 12,100 km^2, and 50,000 km^2 provides a better taxonomic representation coupled with potentially more reliable estimates of the lambda parameter in our phylogenetic least squares regression models (Symonds and Blomberg 2014). We analyze all available data (i.e., regardless of range size) in supplementary analyses and in analyses considering mode of respiration (because few species with small range sizes are available for this particular analysis). Ultimately, the range size used in our analyses appears to be of little concern because our results are generally consistent across the range sizes examined.

General Phylogenetic Least Squares Regression (PGLS) and Model Selection Procedures

We used PGLS (Martins and Hansen 1997) to estimate the relationship between temperature and size traits, using the phylogeny provided by Pyron and Wiens (2013; figs. A1, A2, available online). All life-history and size data were log-transformed before analysis, and all analyses were performed using R (R Development Core Team 2015) by implementing the packages ape (Paradis et al. 2004), phytools

Table 1: Summary of statistical analyses performed

Prefix, range (km^2), and order	Families (%)	*N*		TDOL hypothesis supported		Tables
		Exotrophic	DD	$T_{\max}$	T_{mean}	
EXONEG:						
12,100:						
Anura	33	61		Yes	Yes	A2, A3
Caudata	50	22		Yes	No	A4, A5
50,000:						
Anura	42	118		Yes	Yes	A2, A3
Caudata	70	42		Yes	Yes	A4, A5
All data:						
Anura	71	491		Yes	Yes	A2, A3
Caudata	100	120		No	No	A4, A5
AQTERR:						
12,100:						
Anura	27	55		Yes	Yes	A6, A7
50,000:						
Anura	36	108		Yes	Yes	2, A6, A7
All data:						
Anura	69	440		Yes	Yes	A6, A7
DMI:						
12,100:						
Anura	40	61	21	Yes	Yes	A8–A11
Caudata	50	22	14	Yes	No	A8–A11
50,000:						
Anura	49	118	31	Yes	Yes	3, A8–A11
Caudata	70	42	24	Yes	No	3, A8–A11
All data:						
Anura	80	491	67	Yes	Yes	A8–A11
Caudata	100	121	38	No	No	A8–A11
PATH:						
50,000:						
Anura	49	111	31	Yes	No[a]	A12, A13
Caudata	70	35	22	Yes	Yes	A12, A13
PVI:						
All data:						
Anura	13	100		Yes	No[a]	4, A14, A15
Caudata	60	52		Yes	No[a]	4, A14, A15
Latitude:						
50,000:						
Anura	42	118		Yes		A16
Caudata	70	42		Yes		A16

Note: Analyses include the location of the relevant results tables and whether the analysis supported the temperature-dependent oxygen limitation (TDOL) hypothesis when using mean maximum monthly temperature ($T_{\max}$) or mean monthly temperature (T_{mean}) in the analysis. Families indicate the percent of known taxonomic families represented in each analysis, collapsed across exotrophic and direct developing (DD) species.

[a] Indicates some support apparent for TDOL but uncertainty in relevant parameter estimates.

(Revell 2012), caper (Orme et al. 2013), and geiger (Harmon et al. 2008). PGLS regression parameters and phylogenetic signal were estimated simultaneously, using Pagel's λ (Pagel 1999). We developed statistical models that represent a priori hypotheses with respect to the effect of temperature on size traits. We evaluated the relative support for our hypotheses using corrected Akaike information criterion–based Akaike weights (w_i), and because most sets of models did not reveal a model that was clearly supported (i.e., $w_i < 0.90$ in most cases), we used model averaging to incorporate model uncertainty into parameter estimation (Johnson and Omland 2004). Support for a given hypothesis was therefore evaluated by examining both w_i—which is the relative likelihood of the model, given the data and the alternative models considered—and the effect sizes of the relevant term(s) across all models, as revealed by model-averaged parameter estimates (Burnham and Anderson 2002). Conditional model-averaged parameter estimates (i.e., considering only the models in which the term appears) were calculated using the AICcmodavg (Mazerolle 2014) and gtools (Warnes et al. 2015) packages. Data were centered (i.e., the mean was subtracted) and then divided by two standard deviations (Gelman 2008) so that effect sizes of explanatory variables could be meaningfully compared (Schielzeth 2010).

We performed separate analyses for the Anura and Caudata. First, we tested the overarching prediction that egg diameter of exotrophic Anura will be negatively related to environmental temperature. We compared seven models featuring all possible combinations of the variables log SVL, egg placement, and maximum temperature, which were allowed to affect egg size in an additive fashion. All of these variables have been previously shown to affect egg size variation at the interspecific level (Bradford 1990; Gomez-Mestre et al. 2012), therefore justifying their inclusion in candidate models. The models were denoted with the prefix EXONEG which emphasizes the analysis of exotrophic species with the prediction that egg size will be negatively related to temperature. These models took the form

$$\text{log egg diameter} = \text{log SVL}, \tag{EXONEG.1}$$

$$\text{log egg diameter} = \text{log SVL} + \text{egg placement}, \tag{EXONEG.2}$$

$$\text{log egg diameter} = \text{egg placement}, \tag{EXONEG.3}$$

$$\text{log egg diameter} = \text{egg placement} + \text{max temperature}, \tag{EXONEG.4}$$

$$\text{log egg diameter} = \text{max temperature}, \tag{EXONEG.5}$$

$$\text{log egg diameter} = \text{log SVL} + \text{max temperature}, \tag{EXONEG.6}$$

$$\text{log egg diameter} = \text{log SVL} + \text{egg placement} + \text{max temperature}, \tag{EXONEG.7}$$

where log egg diameter is the species mean estimate of egg diameter (mm), log SVL is species mean snout to vent length (mm), egg placement refers to the location of the eggs before hatch (aquatic, terrestrial, foam nest, or parental soma), and maximum temperature is the mean maximum monthly temperature across the species' range.

Next, if oxygen limitation occurs primarily at the larval stage, not the egg stage, then the strength of the negative association between temperature and egg diameter should not differ for species with aquatic larvae that lay terrestrial eggs (e.g., on branches overhanging the water or in burrows that flood and wash larvae into permanent water bodies) compared with species with aquatic larvae that lay aquatic eggs. For this analysis, we analyzed only exotrophic species that lay eggs aquatically or terrestrially (i.e., we omitted species that lay eggs in foam nests or that carry eggs on parental tissues). Further, only the Anura could be considered because very few Caudata in our data set featured aquatic larvae and terrestrial eggs. To construct the models, we simply expanded the set of EXONEG candidate models to include two additional models that test the interaction between egg placement (aquatic or terrestrial) and temperature. We differentiate between the EXONEG analysis (which features a different data set) and this analysis by labeling models with the prefix AQTERR to reflect the contrast between aquatic and terrestrial eggs. In addition to the seven EXONEG models listed above (relabeled as AQTERR.1–AQTERR.7 for this analysis), the two additional AQTERR models took the form

$$\text{log egg diameter} = \text{egg placement} \times \text{max temperature}, \tag{AQTERR.8}$$

$$\text{log egg diameter} = \text{log SVL} + \text{egg placement} \times \text{max temperature}. \tag{AQTERR.9}$$

Next, if oxygen limitation occurs in aquatic environments, then the relationship between egg diameter and temperature will be more strongly negative for exotrophic compared with direct developing species because the latter group develops entirely on land. We compared six models that were parameterized on the basis of known relationships between egg diameter and egg placement and between egg diameter and development mode; further, we allowed egg size models to vary in the strength of the relationship between egg size and body size of exotrophic versus direct developing species (Gomez-Mestre et al. 2012). If oxygen limitation occurs in eggs or larvae, then it should be temperature dependent, such that the relationship between temperature and egg diameter will be stronger for aquatic developing versus direct developing species (i.e., an interaction between development mode and temperature); we therefore incorporated this interaction into our models. Models are described with prefix DMI, which is meant to emphasize development mode interaction, the term of interest. These models took the form

$$\text{log egg diameter} = \text{log SVL} + \text{mode} + \text{egg placement}, \tag{DMI.1}$$

$$\text{log egg diameter} = \text{log SVL} \times \text{mode} + \text{egg placement}, \tag{DMI.2}$$

$$\text{log egg diameter} = \text{log SVL} + \text{mode} + \text{egg placement} + \text{maximum temperature}, \tag{DMI.3}$$

$$\text{log egg diameter} = \text{log SVL} \times \text{mode} + \text{egg placement} + \text{maximum temperature}, \tag{DMI.4}$$

$$\text{log egg diameter} = \text{log SVL} + \text{egg placement} + \text{mode} \times \text{maximum temperature}, \quad \text{(DMI.5)}$$

$$\text{log egg diameter} = \text{log SVL} \times \text{mode} + \text{egg placement} + \text{mode} \times \text{maximum temperature}, \quad \text{(DMI.6)}$$

where mode is a categorical variable that describes development mode of larva (i.e., exotrophic or direct developing).

Next, given that egg diameter can be strongly associated with adult size and clutch size (Angilletta et al. 2006), we performed phylogenetic path analysis (von Hardenberg and Gonzalez-Voyer 2013) on egg diameter to elucidate the most likely path through which temperature affects egg diameter. Path models and analyses were performed following Gonzalez-Voyer and von Hardenberg (2014), using the caper package for model fitting, and all path models that we tested had four variables: log SVL, log clutch size, log egg diameter, and maximum temperature (fig. 1*a*). All path models assumed that log SVL has a direct causal effect on log clutch size and on log egg diameter. Then, in separate models, we assumed that log clutch size can affect log egg diameter or vice versa, and a direct effect of maximum temperature was introduced via log SVL, log clutch size, or log egg diameter. The expectation was that the best-supported model would feature a direct effect of maximum temperature on log egg diameter (fig. 1*a*). Because incorporating interaction terms into path analysis is difficult, path models were generated separately for direct developing and exotrophic Caudata and Anura, resulting in a total of four separate path analyses.

Finally, if oxygen limitation occurs in larvae, then exotrophic species featuring lungless larvae should experience stronger temperature-dependent oxygen limitation than species where larvae can breathe aerially before metamorphosis. For this analysis, we leveraged the fact that exotrophic larvae differ in their ability to acquire pulmonary oxygen before metamorphosis. Although pulmonary respiration can come at a cost, by exposing the position of the larva to predators (Feder 1983), it allows the larva to supplement cutaneous and branchial oxygen uptake, thereby partly alleviating oxygen limitation when oxygen is low. Although this feature of amphibian development has not been well studied, it is clear that toads in the genus *Bufo* (Bufonidae) and frogs in the genus *Ascaphus* (Ascaphidae) do not have well-developed lungs before metamorphosis (Wassersug and Seibert 1975). Conversely, Anura in the genus *Rana* (Ranidae), *Xenopus* (Pipidae), *Hymenochirus* (Pipidae), *Pseudacris* (Hylidae), *Euphlyctis* (Dicroglossidae), and *Scaphiopus* (Scaphiopodidae) engage in pulmonary ventilation before metamorphosis (Wassersug and Seibert 1975; Feder 1984; Pronych and Wassersug 1994; Crowder et al. 1998). Among the Caudata, all members of the family Plethodontidae lack lungs at all life stages. Similarly, all species in Rhyacotritonidae and Cryptobranchidae, all species of the genus *Nectrurus* (Proteidae), and a few members of Hynobiidae (*Ranodon sibiricus*, *Onychodactylus* sp.) are lungless or feature vestigial lungs at all life stages. Although the timing of lung development among salamanders and newts with lungs is not well studied, it is well known that *Ambystoma* (Ambystomatidae) larvae are air breathers because even small larvae possess well-developed lungs (Heath 1976; Burggren and Pinder 1991). Species without the ability to ventilate aerially should experience stronger temperature-dependent oxygen limitation in aquatic environments, such that egg size should decline relatively quickly with environmental temperature in these species compared with *Ambystoma* species that can breathe air.

Although the extent of pulmonary development before metamorphosis is known to vary within at least one taxonomic family (Nodzenski et al. 1989), we assumed for this analysis that it is invariant within genus. Without this assumption, only a handful of species would be available for analysis. Indeed, that no member of the genus *Bufo* can ventilate, for example, is a widely held assumption that is based on the examination of many *Bufo* species (Wassersug and Seibert 1975; Ultsch et al. 1999). We used egg diameter as a proxy for larval body size (Wells 2010) to test how temperature is related to body size for larval Anura and for larval Caudata. Four models were developed and fit separately for the Anura and Caudata, and they were named using the prefix PVI, reflecting pulmonary ventilation interaction, the term of interest:

$$\text{log egg diameter} = \text{log SVL} + \text{maximum temperature}, \quad \text{(PVI.1)}$$

$$\text{log egg diameter} = \text{log SVL} + \text{pulmonary ventilation}, \quad \text{(PVI.2)}$$

$$\text{log egg diameter} = \text{log SVL} + \text{pulmonary ventilation} + \text{maximum temperature}, \quad \text{(PVI.3)}$$

$$\text{log egg diameter} = \text{log SVL} + \text{pulmonary ventilation} \times \text{maximum temperature}, \quad \text{(PVI.4)}$$

where pulmonary ventilation describes whether the species can ventilate aerially before metamorphosis (i.e., yes or no).

Results

We focus on analyses of species with a range size of up to 50,000 km^2 or less while using maximum temperature as an estimate of temperature. However, details on all analyses—including those using different maximum range sizes and using mean temperature (instead of maximum temperature)—are presented in table 1. We note that our results are generally consistent regardless of how the data are subsetted by range size (i.e., up to 12,100 km^2, 50,000 km^2, or

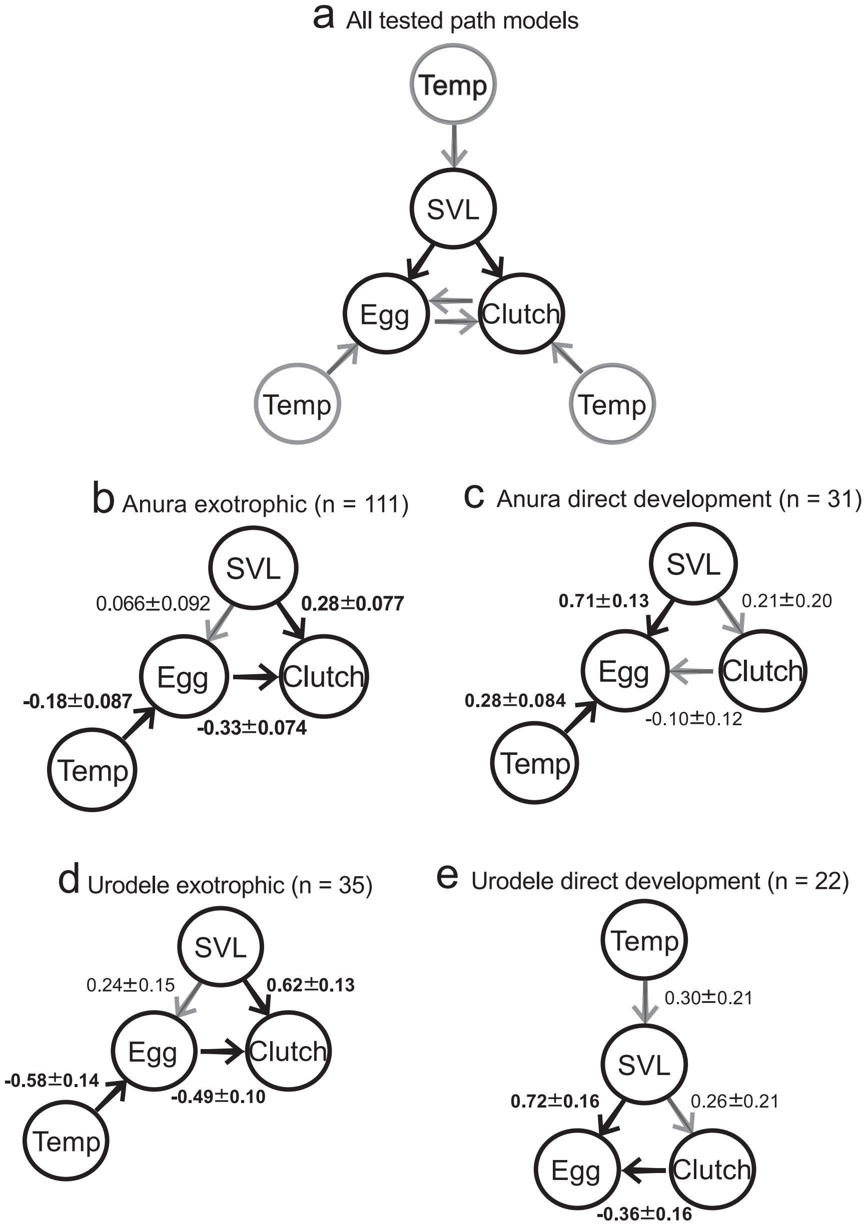

Figure 1: Path models and results, with coefficients (±SE) standardized. *a*, Fixed relationships in all path models are black arrows. Gray arrows represent alternative paths for temperature and alternative paths for the causal relationship between egg diameter and clutch size for a given temperature path (three possible temperature paths × two possible relationships for egg versus clutch size = six different models tested in total, per amphibian group). *b–e*, Best-supported path model for exotrophic Anura (*b*), direct developing Anura (*c*), exotrophic Caudata (*d*), and direct developing Caudata (*e*). In best models (*b–d*), dark arrows and coefficients in bold indicate statistically significant paths, and gray arrows are nonsignificant paths. SVL, snout to vent length.

all available data) especially for the Anura, although for the Caudata support for the relevant predictions declines as the range size of species featured in the analysis increases and when using mean temperature instead of maximum temperature in the analysis (table 1).

We compared PGLS models EXONEG.1–EXONEG.7 to estimate whether the relationship between egg diameter of exotrophs (aquatic larvae) and maximum temperature was negative, considering species with a range size of up to 50,000 km^2. For the Anura ($n = 118$ species), no model was clearly supported (table A2). However, models featuring maximum temperature consistently ranked highly among candidate models, and the model-averaged parameter estimate for maximum temperature was negative and did not

Table 2: Model-averaged parameter estimates and SEs from nine candidate models with prefix AQTERR

Parameter	Estimate	SE
Intercept	.0267	.0828
log SVL	.0691	.0313
Place(terrestrial)	.149	.0355
$T_{\max}$	-.0657	.0319
$T_{\max}$ × place(terrestrial)	.0487	.0534

Note: Models explore whether exotrophic Anura (frogs and toads) laying aquatic eggs ($n = 59$ species) and exotrophic Anura laying terrestrial eggs ($n = 49$ species) show a different relationship between log egg diameter and temperature. Confidence intervals on the interaction between temperature ($T_{\max}$) and egg placement (for place, aquatic is the reference category) exhibit a broad overlap with 0 ($T_{\max}$ × place(terrestrial)), suggesting that species that differ in egg placement (aquatic vs. terrestrial) share a common negative relationship between egg diameter and temperature. Only species with range sizes of up to 50,000 km^2 were considered in the models, and log snout to vent length (SVL) was a covariate in some models. For rankings of all nine candidate models, see table A6, available online.

overlap 0 (table A3). This result was qualitatively consistent whether mean temperature or maximum temperature was used in the analysis (tables A2, A3) and was consistent regardless of the maximum range size of species considered in the analysis (table 1). Further, a contrast among PGLS models AQTERR.1–AQTERR.9 revealed no support for the interaction between egg placement and temperature, suggesting that egg size declines with temperature at a similar rate for exotrophic species that lay eggs on land versus in water (aquatic placement $n = 59$ species, terrestrial placement $n = 49$ species; table 2). A lack of support for the interaction between egg placement and temperature was robust for all subsets of range size and for both mean and maximum temperature (tables A6, A7). We note that the interaction term appears to be weakly supported in some cases (table A6), but the apparent support is erroneous: parameter estimates for the interaction (tables 2, A7) always exhibit a wide overlap with 0, and log likelihoods of the interaction models are very similar to the best model (see Burnham and Anderson 2002, p. 131).

We also compared PGLS models EXONEG.1–EXONEG.7 for the Caudata ($n = 42$ species). As for the Anura, no model was clearly supported, but models featuring maximum temperature always ranked highest, and the model-averaged parameter estimate for maximum temperature was negative and did not overlap 0 (tables A4, A5). This result was consistent when considering only species with very small range sizes, but support for the focal hypothesis was not consistent when considering all species (i.e., regardless of range size) and when mean temperature (instead of maximum temperature) was used in the analysis (tables 1, A4, A5).

Next, we compared PGLS models DMI.1–DMI.6 to estimate whether the negative relationship between temperature and egg diameter is stronger for species with exotrophic larvae (i.e., aquatic larvae) compared with direct developing species (i.e., terrestrial eggs, no aquatic larvae). We identified 31 Anura with direct developing eggs and 118 species with exotrophic larvae, all with a range size of <50,000 km^2. The model featuring an interaction between development mode (i.e., direct developing vs. exotrophic species) and maximum temperature was overwhelmingly supported (model DMI.6, $w_i = 0.90$; table 3). The model-averaged interaction term

Table 3: Rankings of models predicting variation in log egg diameter of Anura and Caudata that vary in mode of development (exotrophic larvae vs. direct development) and egg placement (aquatic, terrestrial, foam nests, or soma)

Order and model	Structure	K	LL	ΔAICc	w_i	λ
Anura ($N = 149$):						
DMI.6	SVL × mode + place + **$\boldsymbol{T}_{\mathbf{max}}$ × mode**	11	−37.25	0	.90	.63
DMI.5	SVL + place + **$\boldsymbol{T}_{\mathbf{max}}$ × mode**	10	−41.22	5.62	.05	.59
DMI.2	SVL × mode + place	9	−43.09	7.06	.03	.57
DMI.4	SVL × mode + place + $T_{\max}$	10	−42.43	8.02	.02	.56
DMI.1	SVL + mode + place	8	−46.15	10.9	.00	.51
DMI.3	SVL + mode + place + $T_{\max}$	9	−45.72	12.3	.00	.50
Caudata ($N = 66$):						
DMI.6	SVL × mode + **$\boldsymbol{T}_{\mathbf{max}}$ × mode**	8	63.38	0	.41	.31
DMI.5	SVL + **$\boldsymbol{T}_{\mathbf{max}}$ × mode**	7	61.98	.27	.36	.47
DMI.4	SVL × mode + $T_{\max}$	7	61.10	2.05	.15	.60
DMI.3	SVL + mode + $T_{\max}$	6	59.32	3.16	.08	.65
DMI.1	SVL + mode	5	52.05	15.4	0	.72
DMI.2	SVL × mode	6	52.45	16.9	0	.73

Note: Models are for species with range sizes of up to 50,000 km^2, using mean maximum monthly temperature ($T_{\max}$) as an estimate of environmental temperature and log snout to vent length (SVL) as a covariate. AICc, corrected Akaike information criterion; LL, log likelihood. Boldface reflects parameter of interest with respect to the temperature-dependent oxygen limitation hypothesis.

suggested that the relationship between egg diameter and maximum temperature is more negative for exotrophic compared with direct developing species (fig. 2*a*; table A9). In supplementary analyses, models featuring an interaction between egg diameter and temperature were also supported, regardless of which subsets of range size were considered and regardless of whether mean temperature or maximum temperature was used in the analysis (tables 1, A8–A11).

We also compared PGLS models DMI.1–DMI.6 for the Caudata. We identified 24 direct developing and 42 exotrophic Caudata with a range size of <50,000 km^2. We omitted the term "egg placement" from all models (i.e., terrestrial vs. aquatic egg laying) because only five exotrophic species featured terrestrial egg placement, and all direct developing species have terrestrial placement; the inclusion of egg placement in any of the analyses below made no qualitative difference to the results (not shown). The two best-supported models were the only two models that featured an interaction between development mode and maximum temperature (models DMI.5 and DMI.6, cumulative $w_i = 0.72$; table 3). As expected, the model-averaged parameter estimate on the interaction term suggests that exotrophic species exhibited a relatively negative relationship between egg diameter and maximum temperature compared with direct developing species (table A9; fig. 2*b*). This result was consistent when considering only species with very small range sizes (tables A8, A9), but support for the interaction term was not apparent when using all available data (i.e., regardless of range size) or when using mean temperature instead of maximum temperature in the analysis (tables A10, A11).

Next, we performed phylogenetic path analysis to identify whether maximum temperature has a direct versus indirect effect on egg-size evolution for species with range sizes of up to 50,000 km^2. For the exotrophic Anura ($n = 111$ species), path analysis revealed support for two competing models (cumulative $w_i = 0.91$; table A12), both of which supported a direct and negative effect of temperature on egg diameter (fig. 1), with the models differing only in the direction of the causal arrow between clutch size and egg diameter (table A12). For exotrophic Caudata ($n = 35$), path analysis revealed support for a model with a direct and negative effect of temperature on egg diameter ($w_i = 0.80$; fig. 1; table A12). For the direct developing Anura ($n = 31$ species),

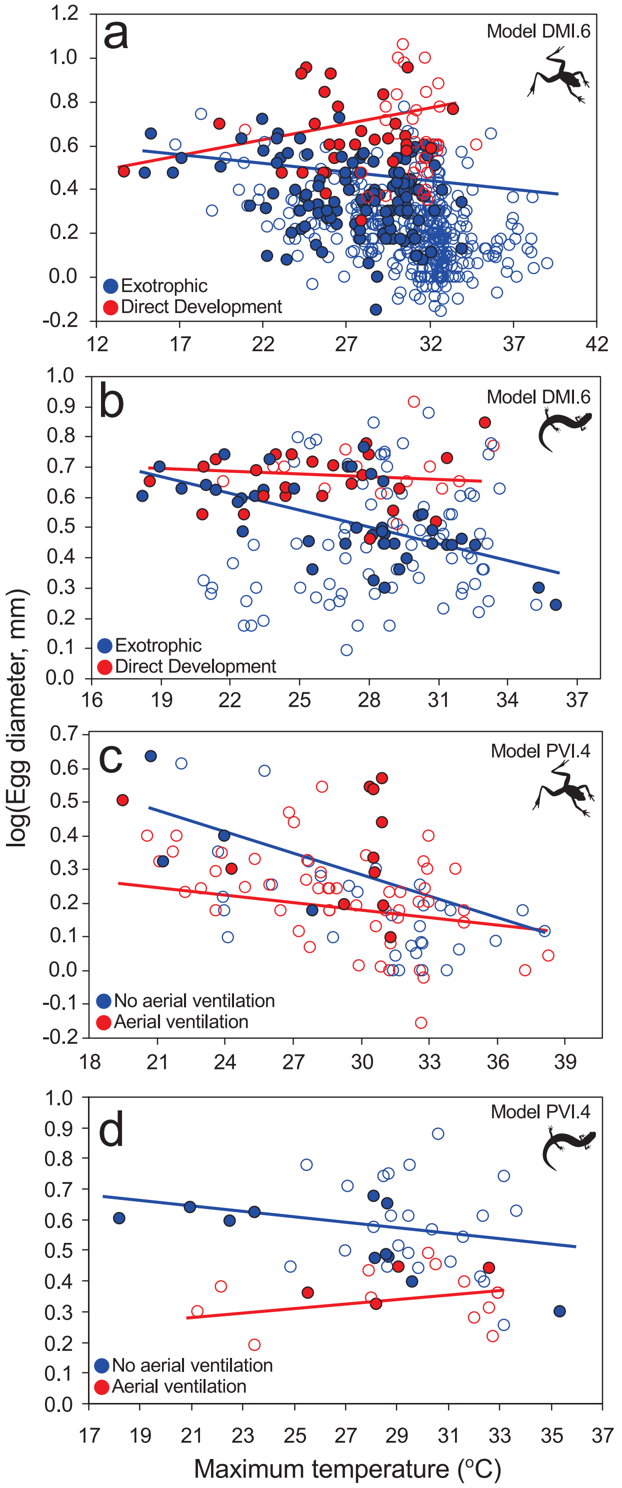

Figure 2: Relationship between mean maximum monthly temperature and egg diameter for species with a range size of up to 50,000 km^2 (filled circles). All available data (open circles) are also plotted for illustrative purposes. For all panels, phylogenetic least squares regression (PGLS) fits pass through phylogenetic means (intercepts), not data means, and raw data (e.g., uncorrected for adult body size) are plotted alongside the line of best fit from PGLS models. *a*, *b*, Egg diameter of Anura (*a*) and Caudata (*b*) for exotrophic (aquatic larvae) species and for species with direct development (terrestrial embryos, no larvae). *c*, *d*, Egg diameter of Anura (*c*) and Caudata (*d*) for species whose larvae are not able to ventilate aerially before metamorphosis and for species whose larvae have well-developed lungs and can ventilate. Because of low availability of data for species with a range size of <50,000 km^2, fitted PGLS regression parameters for *c* and *d* leverage all available data (i.e., including open circles).

path analysis supported two competing models (cumulative $w_i = 1.0$; table A10), both of which featured a direct and positive path between temperature and egg diameter. Finally, for direct developing Caudata ($n = 22$ species), no significant temperature paths were detected (table A12; fig. 1). Using mean temperature instead of maximum temperature in our path analysis suggested support for a direct and negative relationship between mean temperature and egg diameter of exotrophic Anura and Caudata, although model selection uncertainty was greater (i.e., maximum $w_i \leq 0.35$), and for the Anura, the confidence intervals on the parameter estimate indicated uncertainty in the relationship (table A13). More broadly, path models were consistent with well-established correlations among life-history traits and supported a positive relationship between SVL and clutch size for exotrophic amphibians, a positive relationship between SVL and egg diameter for direct developing amphibians, and a negative correlation between egg diameter and clutch size (fig. 1; tables A12, A13).

Finally, we compared models PVI.1–PVI.4, which further explore whether temperature-dependent oxygen limitation might be occurring at the larval stage. We identified 36 species of exotrophic Anura that feature poorly developed lungs before metamorphosis (*Bufo* sp. = 34, *Ascaphus* sp. = 2) and 64 species that can ventilate aerially (*Rana* sp. = 51, *Xenopus* sp. = 1, *Hymenochirus* sp. = 1, *Scaphiopus* sp. = 2, *Pseudacris* sp. = 7, *Euphlyctis* sp. = 2). Few of these species featured range sizes of <50,000 km^2, so we focus on the analysis of all available data, regardless of range size. Using all available data ($n = 100$ species), the model featuring an interaction between maximum temperature and pulmonary ventilation was the best-supported model ($w_i = 0.68$; table 4), with four times greater support than the next-best candidate model ($w_i = 0.17$; table 4). Further, as expected, the interaction between mode of respiration and maximum temperature suggested a relatively strong decline in egg diameter for species that do not ventilate before metamorphosis (fig. 2*c*), and confidence intervals on the interaction term did not overlap 0 (table A14). Only weak support for the interaction term was apparent when using mean temperature (instead of maximum temperature) in this analysis (tables A14, A15): although the best-supported model ($w_i = 0.65$) featured the interaction term (table A15), confidence intervals on the interaction term suggested uncertainty in this relationship (table A14).

We also compared models PVI.1–PVI.4 for the Caudata. We identified 36 species of exotrophic species that are lungless or have vestigial lungs, mainly from the family Plethodontidae (*Desmognathus* sp. = 10, *Eurycea* sp. = 9, *Pseudotriton* sp. = 2, *Gyrinophilus* sp. = 1, *Hemidactylium* sp. = 1, *Stereochilus* sp. = 1), Proteidae (*Necturus* sp. = 3), Hynobiidae (*Ranodon* sp. = 1, *Onychodactylus* sp. = 2), Rhyacotritonidae (*Rhyacotriton* sp. = 3), and Cryptobranchidae (*Andrias* sp. = 2, *Cryptobranchus* sp. = 1). Further, we identified 16 species of *Ambystoma*, whose larvae can ventilate aerially. Like the Anura, this analysis could not be subsetted by range size because few species with small range sizes were available; we therefore used all species in our analysis regardless of range size. Overall, results were very similar to those for the Anura (fig. 1*d*), where we found that support for the model featuring an interaction between maximum temperature and pulmonary ventilation was not overwhelming ($w_i = 0.65$; table 4) but was nevertheless 2.5 times greater than support for the next-best model ($w_i = 0.26$; table 4). Further, the interaction between mode of respiration and maximum temperature suggested a relatively strong decline in egg diameter for species that do not ventilate before metamorphosis (fig. 2*d*), and confidence intervals on the interaction term did not overlap 0 (table A14).

Discussion

This study helps clarify whether the evolution of investment per offspring is constrained by oxygen availability in aquatic

Table 4: Rankings of models predicting variation in log egg diameter of Anura and Caudata with larvae that differ in their ability to engage in aerial pulmonary ventilation before metamorphosis

Order and model	Structure	K	LL	ΔAICc	w_i	λ
Anura:						
PVI.4	SVL + **ventilation** × $\boldsymbol{T_{\max}}$	7	75.34	0	.68	.75
PVI.1	SVL + $T_{\max}$	5	71.64	2.82	.17	.73
PVI.3	SVL + ventilation + $T_{\max}$	6	72.66	3.05	.15	.71
PVI.2	SVL + ventilation	5	62.61	20.9	0	.76
Caudata:						
PVI.4	SVL + **ventilation** × $\boldsymbol{T_{\max}}$	7	63.06	0	.65	.31
PVI.2	SVL + ventilation	5	59.54	1.80	.26	.49
PVI.3	SVL + ventilation + $T_{\max}$	6	59.68	4.08	.08	.44
PVI.1	SVL + $T_{\max}$	5	55.17	10.5	0	.77

Note: Models use mean maximum monthly temperature ($T_{\max}$) as a measure of environmental temperature and log snout to vent length (SVL) as a covariate. AICc, corrected Akaike information criterion; LL, log likelihood.

environments. We demonstrated that in two major clades of amphibians, species mean egg diameter is a significant and decreasing function of environmental temperature for species that feature aquatic larvae (i.e., exotrophic species), and phylogenetic path analysis suggested that temperature is directly influencing this negative relationship. Next, drawing on the well-supported assumption that oxygen limitation is relatively pronounced in aquatic versus terrestrial environments and that oxygen limitation is particularly severe in warm aquatic environments, we went on to provide two lines of evidence that oxygen limitation at the larval stage plays a role in generating the negative relationship between egg size and temperature. First, for both the Anura and the Caudata, the slope of egg diameter over temperature is negative for exotrophic species but positive or neutral for species laying terrestrial eggs that develop directly into juveniles (i.e., direct developing species). Further, the negative relationship between temperature and egg size of exotrophic species seems to arise as a result of factors associated with the larval stage, not the egg stage, as we found that the negative slope of egg diameter over temperature is not different for exotrophic species that lay eggs in water versus exotrophic species that lay eggs on land. Taken together, these findings are consistent with the interpretation that lower investment per offspring evolves when larvae, not embryos, are oxygen limited. The second line of evidence implicates oxygen limitation at the larval stage more directly, as we found that the relationship between egg diameter and temperature was more strongly negative for exotrophic species that are lungless before metamorphosis compared with exotrophic species that develop lungs and breathe aerially before metamorphosis. This finding suggests that when larvae are predisposed to oxygen limitation, low investment per offspring evolves more readily as the environment decreases in oxygen availability. More broadly, by demonstrating that larval size may be constrained by oxygen availability, our results represent an important step toward understanding the evolution of investment per offspring in aquatic environments.

Models of egg size evolution have suggested that large eggs are relatively oxygen limited in aquatic environments (Krogh 1959; Seymour and Bradford 1995; Woods 1999), particularly when flow rates are low (Martin et al. 2017). Yet to our knowledge, there is no direct experimental evidence to suggest that larger embryos are more oxygen limited than small embryos in amphibians or fishes; in fact, the experimental evidence generally favors the opposite view that large embryos perform better under oxygen limitation (Rombough 1989, 2007; Einum et al. 2002; see also Braga Goncalves et al. 2015). This study suggests that oxygen limitation is not related to egg size per se but that oxygen limitation occurs in larvae and is a function of their size, which is related to egg size. Thus, the results are consistent with the hypothesis that maternal investment per offspring is constrained by oxygen limitation in aquatic anamniotes. This is a new view and one that remains compatible with negative correlations between egg size and habitat temperature that are commonly found in the literature (Bradford 1990; Thatje and Hall 2016; Martin et al. 2017). We did not demonstrate precisely how oxygen limitation affects larvae, but the constraint on size likely arises from a confluence of the relatively low oxygen solubility and diffusion coefficient in water. Larvae are small and are dominated by viscous forces that are energetically expensive to overcome, such that ventilation of tissues cannot indefinitely compensate for the higher oxygen demand associated with larger sizes (Atkinson et al. 2006). Oxygen limitation may also be associated with underdeveloped respiratory features in early life, such as gills (Burggren and West 1982; Rombough 2007), resulting in diffusive cutaneous oxygen uptake as a primary means of sustaining aerobic activity during a life stage where predation risk is high.

Patterns of microevolution, macroevolution, and phenotypic plasticity of size in response to temperature are often negative (Moran and Woods 2012; Verberk and Atkinson 2013; Horne et al. 2015), suggesting a unifying explanation for temperature-size relationships (Atkinson 1994; Atkinson and Sibly 1997; Angilletta and Dunham 2003). If temperature-dependent oxygen limitation provides a general explanation for this pattern, then body size of juveniles and adults should show similar negative responses to temperature—and they do, to the extent that the pattern has been dubbed the temperature-size rule (Atkinson 1994; Atkinson et al. 2001). Further, both propagule size and adult size should also show a relatively strong decrease in size in aquatic compared with terrestrial environments (Atkinson et al. 2006). Yet in a meta-analysis of propagule size-temperature responses, Forster et al. (2011, 2012) found no support for the latter prediction, such that their results are difficult to reconcile with the generality of temperature-dependent oxygen limitation. Forster et al.'s (2011, 2012) results are particularly surprising because aquatic juveniles should be susceptible not only to oxygen limitation at high temperature but also to temperature-induced increases in water viscosity at low temperature, where drag forces in water become increasingly energetically expensive to overcome with smaller size, disproportionately limiting oxygen regulation in early life stages (Moran and Woods 2012; Verberk and Atkinson 2013). Notably, Forster et al. (2012) focused primarily on changes in adult size with temperature, whereas changes in propagule size with temperature seemed to be an ancillary component of their study, and their sample size for propagule size was far smaller than for adult traits. Further, as Klok and Harrison (2013) point out, Forster et al. (2012) also combined data from diverse groups (reptiles, amphibians, and fish, but primarily insects) into a single analysis and converted all sizes to dry weights, all of which may have introduced additional uncertainty into their analysis of propagule size, especially given the small sample size. Although our results are essentially in contrast to those of Forster et al. (2012), ours help reconcile the general observation that adult size and propagule size respond independently to temperature both within species (Atkinson et al. 2001) and across species (Moran and Woods 2012), and our results are consistent with both verbal and quantitative theories of temperature-dependent oxygen limitation (Makarieva et al. 2005; Atkinson et al. 2006).

Our study relies on correlations between habitat and temperature, and although we interpret our findings in terms of oxygen limitation, we did not measure oxygen availability. Different interpretations are therefore possible. For instance, seasonality becomes stronger as latitude increases, and both theory (Rowe and Ludwig 1991; Abrams and Rowe 1996) and empirical work (Johansson and Rowe 1999; Lee et al. 2012) suggest selection for increased growth rates in time-constrained environments. The costs of accelerated growth during early life may be alleviated by high investment in individual offspring (Eckerstrom-Liedholm et al. 2017), such that the negative associations between egg size and temperature observed in this study could ultimately be attributable to time constraints. Yet this is unlikely because even after accounting for latitude in this study, the negative association between temperature and egg size of exotrophic species persists (table A16). Other spatial factors may have nevertheless influenced our results. For example, our simulation suggests that annual temperature averages likely capture some of the variation in larval temperatures across a large geographic gradient (table A1), but our simulation did not consider systematic variation in the timing and duration of reproduction across latitude. Although larval duration depends on many factors, such as size at metamorphosis and hydroperiod (Edge et al. 2016), it is possible that long larval periods generally occur in cool environments during the summer, whereas short larval periods occur in warm environments during the winter. This type of systematic geographic variation may have acted to reduce the accuracy by which annual temperature averages map onto the temperatures experienced during the larval period. Future studies should therefore attempt to obtain more precise estimates of the temperatures that are relevant to larval biology.

A different class of explanation for our results relate to intrinsic differences in the environments that are inhabited by the amphibian groups that we attempted to contrast in our analyses. Among salamanders, for example, lungless forms with aquatic larvae often inhabit streams, whereas lunged salamanders are more common in pools, lakes, and ponds (Petranka 1998; Sparreboom 2014). Lungless salamanders that breed in streams feature very large eggs and larvae, and although this pattern may be facilitated by relatively high oxygen availability in streams (Beachy and Bruce 1992), it may ultimately result from the larger size of prey items in streams compared with ponds (Nussbaum 1987). With respect to this study, then, there are fundamental and unexpected differences in the environments and groups that we attempted to contrast, and correlations between egg size and temperature might not reflect oxygen limitation. For instance, the stronger temperature-related decline in egg size that we observed among lungless amphibian larvae compared with lunged forms (fig. 2*c*, 2*d*) may be the result of adaptation to differences in how stream versus pond communities assemble with increasing temperature and not necessarily the result of temperature-dependent oxygen limitation. Thus, while our results are consistent with oxygen limitation at the larval stage, we underline that our analysis is based on correlations, and many features of the environments we compared—not just oxygen levels—likely exhibit different responses to temperature, potentially undermining support for the oxygen limitation hypothesis.

In sum, classical work suggested that surface area to volume relationships coupled with oxygen diffusion rates constrain the evolution of large egg size in aquatic environments and hence the evolution of investment per offspring (Krogh 1959). The weight of evidence now suggests that egg size per se is unlikely to influence oxygen availability to embryos (Rombough 1989, 2007; Einum et al. 2002; Braga Goncalves et al. 2015), which implies that the evolution of investment per offspring is not constrained by oxygen limitation. Yet larval body size is directly related to egg size and hence investment per offspring, especially in amphibians (Wells 2010, p. 499), and oxygen relations after hatching do not necessarily mirror those during the egg stage (Rombough 2007). To date, there has been contrasting evidence that investment per offspring is influenced by temperature-dependent oxygen limitation (Atkinson et al. 2001; Forster et al. 2012), or the evidence has been equivocal by virtue of methodological limitations imposed by the study system (Bradford 1990; Fleming and Gross 1990; Feiner et al. 2016; Thatje and Hall 2016). Our study helps clarify this picture because we leverage an ecologically diverse group of vertebrates and provide evidence that temperature-dependent oxygen limitation affects the evolution of investment per offspring by virtue of oxygen relations that occur during the larval stage.

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Green frog (*Lithobates clamitans*). Gouache and art marker. By Melanie Massey, 2016.