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ORIGINAL PAPER

Eye-body allometry across biphasic ontogeny in anuran amphibians

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Abstract

Animals with biphasic lifecycles often inhabit different visual environments across ontogeny. Many frogs and toads (Amphibia: Anura) have free-living aquatic larvae (tadpoles) that metamorphose into adults that inhabit a range of aquatic and terrestrial environments. Ecological differences influence eye size across species, but these relationships have not yet been explored across life stages in an ontogenetic allometric context. We examined eye-body size scaling in a species with aquatic larvae and terrestrial adults, the common frog *Rana temporaria*, using a well-sampled developmental series. We found a shift in ontogenetic allometric trajectory near metamorphosis indicating prioritized growth in tadpole eyes. To explore the effects of different tadpole and adult ecologies on eye-body scaling, we expanded our taxonomic sampling to include developmental series of eleven additional anuran species. Intraspecific eye-body scaling was variable among species, with 8/12 species exhibiting a significant change in allometric slope between tadpoles and adults. Traits categorizing both tadpole ecology (microhabitat, eye position, mouth position) and adult ecology (habitat, activity pattern) across species had significant effects on allometric slopes among tadpoles, but only tadpole eye position had a significant effect among adults. Our study suggests that relative eye growth in the preliminary stages of biphasic anuran ontogenies is somewhat decoupled and may be shaped by both immediate ecological need (i.e. tadpole visual requirements) and what will be advantageous during later adult stages.

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Introduction

Eye size is an important determinant of visual function in vertebrates, with larger eyes typically associated with better visual sensitivity and resolution. However, eyes are metabolically expensive, and thus eye size reflects the trade-offs between visual needs and metabolic costs (Niven and Laughlin 2008). Variation in ontogenetic eye-body allometry therefore likely evolves as a consequence of different optimal growth patterns in different environments (Hutton and McGraw 2016) and/or to support different visual needs during particular developmental stages (Gisbert 1999). For example, in fishes, inflection points in ontogenetic eye-body allometry coincide with the transition from yolk sac nourishment to independent feeding. Larval fishes tend to have a positive eye-body allometry, whereas later stages have isometric or negative allometries. This pattern has been interpreted as evidence of prioritized larval eye development to facilitate adult feeding behaviours that are dependent on vision (Gisbert 1999; Saemi-Komsari et al. 2018). Ontogenetic shifts in eye-body allometries are likely widespread among vertebrates, but they have only been examined in a handful of species that largely inhabit spectrally similar environments throughout ontogeny (e.g., fishes: Gisbert 1999; Moshayedi et al. 2015; Saemi-Komsari et al. 2018; geckos: Werner and Seifan 2006). In many vertebrates, development occurs across diverse environments, where the light regime, visual requirements, resource availability, and even the optical medium through which vision occurs (water vs. air) can vary drastically at different stages of ontogeny. Species that have biphasic life cycles with major ecological shifts at metamorphosis represent an ideal scenario for exploring ontogenetic shifts in eye-body allometry and understanding the extent to which ecology influences ontogenetic eye scaling.

Many anuran amphibians (Amphibia: Anura = frogs and toads) experience a major ontogenetic transition as they metamorphose from aquatic larvae (tadpoles) to terrestrial adults. Vision is a key sensory system for most adult anurans, which have large relative eye sizes and a high slope for evolutionary eye-body allometry among major vertebrate groups (Thomas et al. 2020). Likewise, vision is important to aquatic tadpoles for locating food, avoiding predation, and recognizing conspecifics (McDiarmid and Altig 1999). Finally, there is evidence that morphological evolution in tadpoles and adults is decoupled to some extent (Sherratt et al. 2017; Valero et al. 2017; Phung et al. 2020). Despite the ostensible importance of vision throughout the life-history of many anurans and the extensive ecological diversity of this group, it is unclear if eye growth is prioritised during larval stages (as in some fishes), prioritised in adults, or varies with phylogeny or ecology (Thibaudeau and Altig 2012). Although several studies have examined relative eye growth in larval anurans (e.g., de Jongh 1967; Hall et al. 1997), corresponding data on adult eye scaling is needed to understand eye-body allometry across anuran biphasic ontogeny.

In this study we address four main questions: Is ontogenetic eye-body allometry decoupled between larval and adult anurans? If anurans exhibit ontogenetic shifts in allometry, is there a higher investment in eye growth in tadpoles or adults? Is eye-body allometry across species more conserved among tadpole or adult stages? If there is variation among species, do allometric differences correlate with adult and/or tadpole ecology? We predict that (1) anurans exhibit shifts in ontogenetic allometry at metamorphosis; (2) anuran eye-body scaling exhibits higher slopes in tadpoles than adults to facilitate large adult eye sizes; (3)

tadpoles inhabiting similarly aquatic habitats will exhibit more conserved allometries than adults inhabiting a wider range of aquatic and/or terrestrial habitats; and (4) allometric differences among species correlate with differences in tadpole ecology (microhabitat, eye and mouth position) and adult ecology (microhabitat, activity period) relevant to vision.

We first investigate ontogenetic eye-body allometry across metamorphosis with a high-resolution growth series of the European common frog, *Rana temporaria* Linnaeus, 1758, a species with aquatic, predominantly benthic larvae and predominantly terrestrial, ground-dwelling adults. We then broadened our sampling with partial ontogenetic series for an additional eleven species – *Leptopelis spiritusnoctis* Rödel, 2007, *Trichobatrachus robustus* Boulenger, 1900, *Bufo bufo* (Linnaeus, 1758), *Hemisus marmoratus* (Peters, 1854), *Hyla meridionalis* Boettger, 1874, *Pseudis paradoxa* (Linnaeus, 1758), *Kaloula pulchra* Gray, 1831, *Microhyla rubra* (Jerdon, 1853), *Xenopus victorianus* Ahl, 1924, *Aubria subsigillata* (Duméril, 1856), and *Polypedates leucomystax* (Gravenhorst, 1829) – to compare ontogenetic allometry across a broad taxonomic and ecological diversity of anurans.

Materials and methods

Sampling strategy

True ontogenetic eye-body allometry is the relationship between eye size and body size in a single individual throughout all developmental stages (Gould 1966). However, this can be approximated by sampling many individuals of a species in different stages of development, and this was our approach. We first generated a high-resolution ontogenetic series of *Rana temporaria* using preserved specimens from the Natural History Museum, London (specimen numbers with BMNH prefix; Appendix 1). We selected this species for high-resolution sampling based on (1) its ecological transition from aquatic to terrestrial habitats at metamorphosis and potential for exhibiting an ontogenetic shift in eye-body allometry, and (2) the availability of specimens ($N=152$) from all post-hatching Gosner (1960) stages (see below for more detail on developmental staging) and broad range of both subadult and mature adult body sizes.

We then generated lower-resolution growth series data for 11 other species of anuran amphibians with aquatic larvae, from eight additional families (Appendix 1). Sampling was targeted at maximising phylogenetic and ecological diversity (Table 1), but limited by the availability of specimens in collections, because tadpoles of diverse developmental stages as well as adults of varying body sizes were required to generate reasonable allometric fits. We sampled *Xenopus victorianus* ($N=69$), *Bufo bufo* ($N=75$), *Hyla meridionalis* ($N=50$), *Pseudis paradoxa* ($N=52$), *Aubria subsigillata* ($N=31$), *Polypedates leucomystax* ($N=60$), *Kaloula pulchra* ($N=39$), *Microhyla rubra* ($N=45$), *Hemisus marmoratus* ($N=51$), *Leptopelis spiritusnoctis* ($N=44$), and *Trichobatrachus robustus* ($N=55$). We supplemented BMNH specimen data using specimens from Museum für Naturkunde (ZMB; Berlin, Germany). All specimens measured for this study are listed in the Appendix.

Morphological measurements

Standardised anuran morphological measurements were taken as outlined by Watters et al. (2016) for adults and McDiarmid and Altig (1999) for tadpoles (Fig. 1). Three measures were recorded across all specimens: transverse eye diameter, snout–vent length (SVL), and

Table 1 Twelve species of frogs and toads (Amphibia: Anura) included in this study

Taxon	Adult habitat	Binary adult habitat	Adult activity period	Tadpole microhabitat	Tadpole mouth position	Tadpole eye position	Geographical origin
Ranidae							
Rana temporaria	Ground	Other	Both	Benthic	Anteroventral	Dorsal	Europe
Pipidae							
Xenopus victorianus	Aquatic	Aq/Foss	Nocturnal	Nektonic	Terminal	Lateral	Africa
Bufonidae							
Bufo bufo	Ground	Other	Nocturnal	Benthic	Anteroventral	Lateral	Europe
Hylidae							
Hyla meridionalis	Scansorial	Other	Both	Benthic	Anteroventral	Lateral	Europe
Pseudis paradoxa	Aquatic	Aq/Foss	Both	Nektonic	Anteroventral	Lateral	S. America
Pyxicephalidae							
Aubria subsigillata	Aquatic	Aq/Foss	Nocturnal	Benthic	Anteroventral	Dorsal	Africa
Rhacophoridae							
Polypedates lecuomystax	Scansorial	Other	Nocturnal	Benthic	Anteroventral	Lateral	Asia
Microhylidae							
Kaloula pulchra	Subfossorial	Aq/Foss	Nocturnal	Nektonic	Terminal	Lateral	Asia
Microhyla rubra	Subfossorial	Aq/Foss	Nocturnal	Nektonic	Terminal	Lateral	Asia
Hemisotidae							
Hemisus marmoratus	Fossorial	Aq/Foss	Nocturnal	Nektonic	Anteroventral	Lateral	Africa
Arthroleptidae							
Leptopelis spiritusnoctis	Scansorial	Other	Nocturnal	Benthic	Anteroventral	Dorsal	Africa
Trichobatrachus robustus	Semiaquatic	Other	Nocturnal	Benthic	Ventral	Dorsal	Africa

We sampled a near complete developmental series for *R. temporaria* and partial developmental series in the other species. For each species, we investigated eye growth trajectories across their biphasic ontogeny. Natural history, ecological, and morphological traits relevant to visual ecology are listed for each species. Note that adult habitat, adult activity period, and tadpole microhabitat refer to predominant associations and activities. Aq/Foss = aquatic or fossorial

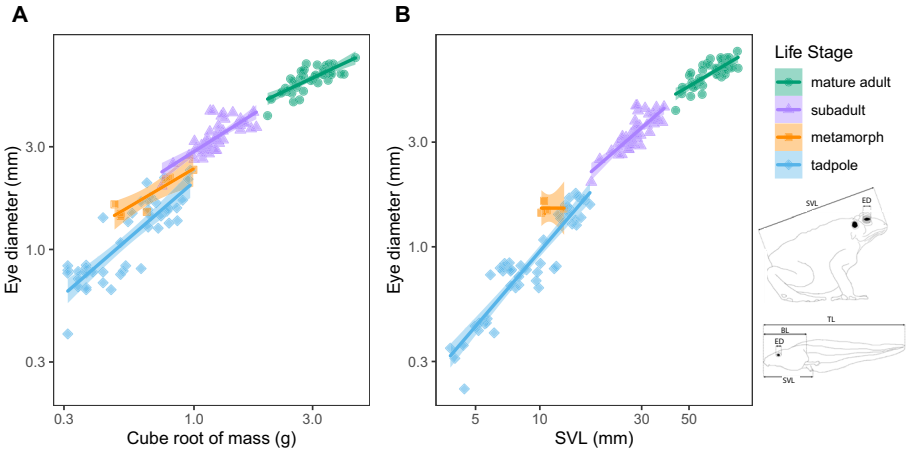

Fig. 1 Eye-body size allometry across ontogeny in the common frog, *Rana temporaria*. Four broad developmental stages (adult, subadult, metamorph, and tadpole) are indicated by coloration and symbol. Lines are coloured by life stage and show OLS regressions with 95% confidence intervals for $\log_{10}$ eye diameter vs. $\log_{10}$ the cube root of mass (**a**) or $\log_{10}$ SVL (**b**) for each stage. Larval illustration redrawn from Ariyasiri et al. (2011). Adult illustration redrawn from Watters et al. (2016)

wet mass. Two additional length measurements were taken for tadpoles: total length and body length. Eye diameter was measured as the externally visible portion of the eye, as in Watters et al. (2016). Measurements were taken with digital callipers (to 0.01 mm) or an ocular micrometer (0.01 mm) for small specimens. Total mass was measured using a KERN CM 60-2N pocket balance (0.01 g) for specimens < 60 g and a KERN CM 1K1N pocket balance (1 g) for specimens ≥ 60 g.

For each specimen, measurements were taken from both the left and right eye if possible and averaged prior to analysis. For frogs preserved with a distorted or curved spine, SVL was measured by running a piece of thread along the midline from the tip of the snout to the posterior edge of the vent, then measuring the thread using digital callipers. Specimens exhibiting severe contortions were excluded. Wet mass was recorded within thirty seconds following removal from alcohol after shaking off residual surface liquid. Where specimens were individually tagged with an identification number, the tag mass was subtracted from the total mass.

Preservation is known to alter morphology in amphibians (e.g., Pierson et al. 2020). However, a previous study found little difference in anuran evolutionary eye-body allometry based on fresh vs. preserved specimens and found similar patterns of eye scaling compared to SVL or wet mass (Thomas et al. 2020). Further, our results demonstrate that ontogenetic eye-body allometric slopes show similar patterns using SVL or mass as a measure of body size. Results using both measures of body size are included to demonstrate they do not change our main conclusions.

Developmental staging

Specimens were staged following Gosner's (1960) developmental scale. Specimens spanned the earliest developmental stage with clearly discernible eyes (stage 22) to the completion of metamorphosis (stage 46). For analyses, individuals were divided into

tadpole (stages 22–40), metamorph (stages 41–45), and adult (stage 46) life stages following McDiarmid and Altig (1999). For the more comprehensively sampled *Rana temporaria*, adults (stage 46) were further divided into subadults (12–43 mm SVL, includes juveniles) and mature adults (>43 mm SVL) based on the length frequency distribution generated by Vences et al. (1999). These finer categories were used in initial examination of *R. temporaria* allometry, but the single adult category was used when comparing *R. temporaria* to other species. While we did not classify adults into juveniles/subadults and adults in 11 of the species, we sampled the broadest range of post-metamorphic body sizes available in the collections to ensure we were capturing ontogenetic rather than static allometry in adults.

Data, analysis and reproducibility

All analyses were performed in RStudio 1.2.5033 (RStudio Team 2019) using the statistical programme R 3.6.2 (R Core Team 2019). We used the package plyr v.1.8.6 (Wickham 2011) and package suite tidyverse v.1.3.0 (Wickham et al. 2019) for data manipulation and visualization, and cowplot v.1.0.0 for arranging figure panels (Wilke 2019). The datasets supporting this article have been uploaded to the NHM Data Portal (Shrimpton et al. 2021), and annotated code to reproduce all analyses and quantitative aspects of figures are available on the following GitHub repository: https://github.com/knthomas/anuran-allometry.

Testing for eye-body allometric shifts at metamorphosis

We first investigated whether anurans exhibit shifts in ontogenetic eye-body allometry at metamorphosis using our two measures of body size (mass and SVL). Body mass was converted to the cube root of mass for analyses so that isometry with eye diameter would occur at a slope of one. We used ordinary least squares (OLS) in stats v.3.4.2 (R Core Team 2019) to fit multiple linear regressions of $\log_{10}$ eye diameter vs. $\log_{10}$ the cube root of mass × life stage and of $\log_{10}$ eye diameter vs. $\log_{10}$ SVL × life stage to determine eye-body ontogenetic allometry and test for differences among life stages (tadpoles, metamorphs, adults [further subdivided into subadults and mature adults in *Rana temporaria*]) within each species. We then fit a multiple linear regression of $\log_{10}$ SVL vs. $\log_{10}$ the cube root of mass × life stage to examine how our two measures of body size covaried through ontogeny.

Because literature is divided on whether it is best to use OLS or standardized major axis (SMA) regression for allometry (see Jürgens 1991; Warton et al. 2006; Smith 2009; Kilmer and Rodríguez 2017), we also derived the same allometric relationships using SMA regressions in smatr v.3.4.8 (Warton et al. 2012), which are available in the Supplementary Materials. In SMA regressions, pairwise comparisons of slopes between life stages were adjusted for multiple comparisons with the Šidák correction to control for family-wise error rate (Westfall et al. 2006; Warton et al. 2012).

Comparing ontogenetic eye-body allometry across species

To examine variation in ontogenetic eye-body allometry across species, we used linear mixed models implemented in lme4 v.1.1.25 (Bates et al. 2015) with $\log_{10}$ eye diameter as the response variable, $\log_{10}$ body size (cube root of mass or SVL) as a fixed

effect, and species identity as a random effect. Two models fitted with restricted maximum likelihood (REML) were run separately for tadpoles and adults: a variable intercepts model assuming a common allometric slope among species groups, and a variable slopes model allowing different slopes and intercepts across species, following Firmat et al. (2014). We compared model fits by Akaike's information criterion (AIC).

For comparison, we also tested for differences in ontogenetic allometry among species using SMA regression of eye size vs. body size × species for tadpoles and adults separately. Following this, multiple comparisons with Šidák corrections were used to identify which species differed significantly in slope from one another.

Testing for effects of ecology on eye-body allometry

Finally, we investigated whether allometric slopes for eye-body scaling were associated with adult or tadpole ecology. We assigned each species to discrete ecological trait categories using data from primary literature (Downie et al. 2009), books (McDiarmid and Altig 1999; Channing et al. 2012), and online databases (AmphibiaWeb 2020; IUCN 2020). Previous work has shown that adult habitat has a significant effect on evolutionary eye-body allometry in anurans, and that species inhabiting fossorial, subfossorial, and aquatic habitats generally have smaller relative eye sizes as adults than species that are semiaquatic, ground-dwelling, or scansorial (Thomas et al. 2020). To see how adult habitat may affect ontogenetic allometry, we categorized species as "aq/foss" (aquatic, fossorial, or subfossorial) or "other" (semiaquatic, ground-dwelling, or scansorial) using the same criteria as Thomas et al. (2020). We also categorized adult activity pattern in each species as "nocturnal" (primarily nocturnal activity) or "both" (diurnal and nocturnal activity common) as in Thomas et al. (2020); we had no primarily diurnal species in our dataset. We then assigned species to three ecological and morphological traits associated with tadpole vision and feeding: (1) aquatic microhabitat (benthic or nektonic), (2) position of eyes (lateral or dorsal), and (3) position of mouth (anteroventral, terminal, or ventral). All species in our study have aquatic, free-living, feeding tadpoles.

We then tested for the effects of ecology on eye-body ontogenetic allometry using phylogenetic linear mixed models implemented in MCMCglmm v.2.29 (Hadfield 2010). In these models, we used the phylogeny of Jetz and Pyron (2018), pruned to our 12 focal species in ape v.5.3 (Paradis et al. 2004). We fitted models separately for tadpoles and adults and, to avoid overparameterization, separately for each of the five ecological variables we tested (adult habitat, adult activity pattern, tadpole microhabitat, tadpole eye position, tadpole mouth position). In each model, the response variable was $\log_{10}$ eye diameter, the fixed effects were $\log_{10}$ body size (cube root of mass or SVL) and ecology, and our random effects were species identity and the phylogenetic non-independence among species. We used the default diffuse prior distribution ($\mu = 0$ and $V = 1^{10}$) for fixed effects, and set the prior distribution for random effects using an inverse Wishart of $V = 1$ and $v = 0.02$ (a fairly uninformative prior). We ran each model for 1 million iterations with a burnin of 1000 and sampling interval of 200. We assessed model convergence using standard diagnostic plots, and effective sample sizes exceeded 3000 for all models. We assessed significance of fixed effects by examining the 95% highest posterior density (HPD) intervals and using MCMC p-values (Hadfield 2010).

Results

Eye-body allometry in *Rana temporaria*

Ontogenetic eye-body mass allometry differed significantly among life stages in *Rana temporaria* (Fig. 1a). Log body mass ($F_{(1)}=2922$, $p<0.001$), life stage ($F_{(3)}=33.7$, $p<0.001$), and their interaction ($F_{(3)}=4.0$, $p<0.01$) all had significant effects on log eye diameter in the OLS model. Data were explained well by the model ($F_{(7,129)}=433.5$, $p<0.001$, $R^2_{adj.}=0.96$). Tadpoles exhibited a significantly higher slope ($b=1.00$, SE = 0.15, t = 3.10, $p=0.002$) for eye-body mass allometry than mature adults ($b=0.54$, SE = 0.13). Metamorphs did not differ from mature adults significantly in slope ($b=0.68$, SE = 0.26. t = 0.64, $p=0.59$), but had a significantly lower intercept ($a=0.38$, SE = 0.08, t = −2.06, $p=0.04$). Subadults did not differ from adults in slope ($b=0.73$, SE = 0.18, t = 1.05, $p=0.30$) or intercept ($a=0.45$, SE = 0.06, t = −1.32, $p=0.19$). The SMA model likewise showed that only tadpole and mature adults differed significantly in allometric slopes (Table S2, Fig. S1a).

Eye-body length allometry showed similar trends across life stages in *R. temporaria* (Fig. 1b). Log SVL ($F_{(1)}=5562$, $p<0.001$), life stage ($F_{(3)}=27.1$, $p<0.001$), and their interaction ($F_{(3)}=7.5$, $p<0.001$) each had significant effects on log eye diameter. Data were explained well by the model ($F_{(7,133)}=809.4$, $R^2_{adj.}=0.98$, $p<0.001$). Tadpoles exhibited a significantly higher slope ($b=1.14$, SE = 0.13, t = 4.19, $p<0.001$) and significantly lower intercept ($a=-1.16$, SE = 0.23, t = −3.95, $p<0.001$) than adults ($b=0.57$ SE = 0.13; $a=-0.24$, SE = 0.23), while subadults ($b=0.84$, SE = 0.17; $a=-0.71$, SE = 0.29) and metamorphs ($b=-0.001$, SE = 0.74; $a=0.18$, SE = 0.79) did not exhibit significant differences from adults in eye-body length allometry ($p>0.05$). The SMA model likewise showed a significant difference in allometric slopes between tadpoles and mature adults (Table S5, Fig. S1b).

Ontogenetic shifts in eye-body allometry within species

Results discussed in this section are based primarily on log-transformed OLS comparisons of eye diameter and cube root of mass in each species (Fig. 2; Table 2). Full results for eye diameter and SVL can be found in the supplemental materials (Figs. S3-4; Tables S3-5) along with comparisons of SVL and the cube root of mass (Fig. S5-6, Tables S6-8). We found significant effects of life stage on ontogenetic eye-body allometry in 11/12 sampled species (Fig. 2, Table S1). Of these, eight species – *Rana temporaria*, *Bufo bufo*, *Aubria subsigillata*, *Xenopus victorianus*, *Polypedates leucomystax*, *Leptopelis spiritusnoctis*, *Microhyla rubra*, and *Hemisus marmoratus* – showed a significant difference in intraspecific eye-body allometric slopes among life stages (Fig. 2; Table 2). Three species showed no significant difference in slopes among life stages, but did exhibit significant differences in intercepts: *Pseudis paradoxa*, *Kaloula pulchra*, and *Trichobatrachus robustus*. Only *Hyla meridionalis* exhibited no significant difference in slope or intercept among life stages. In comparisons of eye size with SVL, we found similar evidence of shifts in ontogenetic allometry at metamorphosis in 9/12 species (Table S4, Fig. S3).

We also found that tadpoles tend to invest more in eye growth relative to body growth than adults. Tadpoles had significantly higher slopes than adults in 6/8 of the species with a significant change in slope across life stages (Table 2): *R. temporaria* (1.00 vs.

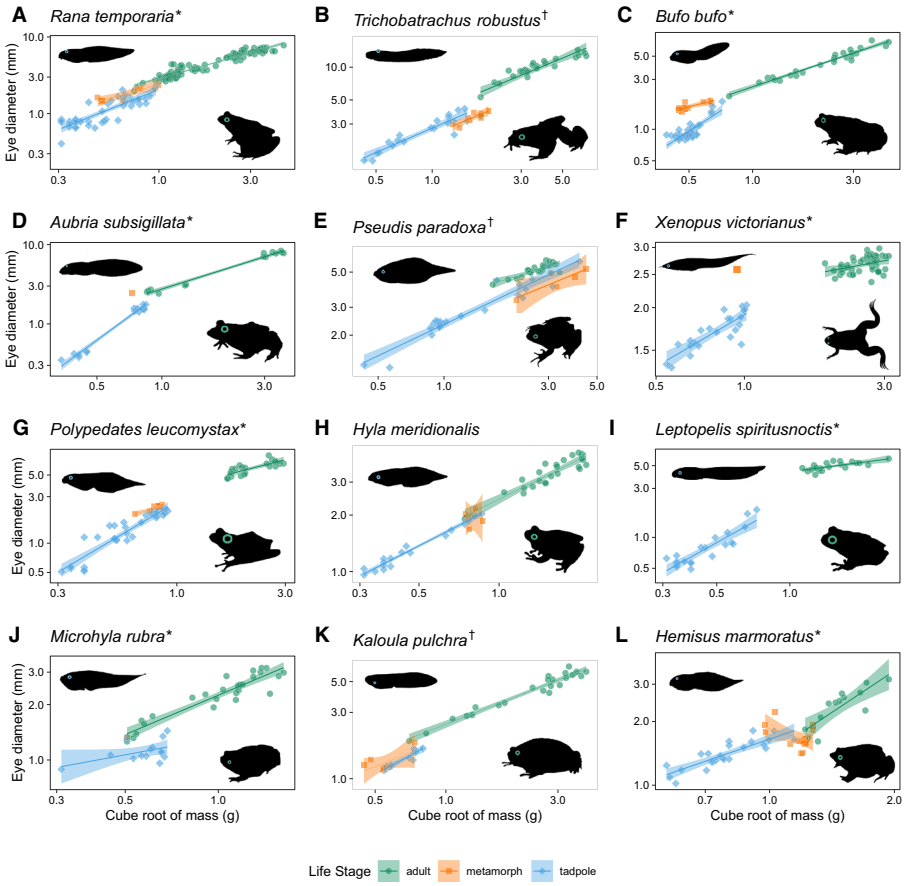

Fig. 2 Eye-body size allometry across ontogeny in 12 species of anuran amphibians: *Rana temporaria* (**a**), *Trichobatrachus robustus* (**b**), *Bufo bufo* (**c**), *Aubria subsigillata* (**d**), *Pseudis paradoxa* (**e**), *Xenopus victorianus* (**f**), *Polypedates leucomystax* (**g**), *Hyla meridionalis* (**h**), *Leptopelis spiritusnoctis* (**i**), *Microhyla rubra* (**j**), *Kaloula pulchra* (**k**), and *Hemisus marmoratus* (**l**). Three life stages (adult, metamorph, and tadpole) are indicated by colouration and symbol. Lines are coloured by life stage and indicate OLS regressions with 95% confidence intervals for $\log_{10}$ eye diameter versus $\log_{10}$ the cube root of mass for each stage. Inset silhouettes depict the relative eye size of each species as tadpoles and adults, respectively. Asterisks indicate species with a significant difference between tadpole and adult slopes. Daggers indicate species with no significant difference between tadpole and adult slopes, but a significant difference in intercepts between life stages

0.71), *X. victorianus* (0.50 vs. 0.16), *B. bufo* (1.30 vs. 0.68), *A. subsigillata* (1.99 vs. 0.85), *P. leucomystax* (1.38 vs. 0.60), and *L. spiritusnoctis* (1.47 vs. 0.43). Only *M. rubra* (0.31 vs. 0.71) and *H. marmoratus* (0.68 vs. 1.30) showed higher slopes in adults. The same pattern was observed in comparisons of eye diameter and SVL; tadpoles had significantly higher slopes than adults in 8/9 species with different slopes among life stages (Table S4).

Metamorphs had low sample sizes in most species (n = 0 to 14), but for species with > 1 metamorph sampled, 6/8 species showed no significant difference in slope or intercept between metamorphs and adults (Table 2). *Hemisus marmoratus* had a significantly

Table 2 Coefficient estimates from OLS multiple linear regressions of $\log_{10}$ mean eye diameter (mm) versus $\log_{10}$ cube root of mass (g×life stage in 12 species of anuran amphibian. *p* values for adults indicate whether coefficients differ significantly from 0; *p* values for other life stages indicate the significance of the contrast with adults for slopes (*b*) or intercepts (*a*)

Taxon	Life stage	*N*	*b* (95 % CI)	t_b	p_b	*a* (95 % CI)	t_a	p_a	Model significance
Rana temporaria	Ad	87	0.71 (0.64, 0.78)	20.2	**< 0.001**	0.46 (0.44, 0.48)	40.1	**< 0.001**	**$p < 0.001$**, $F_{(5,131)} = 607.9$, $R^2_{adj.} = 0.96$
	Meta	7	0.68 (0.23, 1.13)	-0.12	0.91	0.38 (0.28, 0.47)	-1.66	0.10	
	Tad	43	1.00 (0.85, 1.15)	3.88	**< 0.001**	0.31 (0.27, 0.36)	-6.11	**< 0.001**	
Xenopus victorianus	Ad	45	0.16 (0.03, 0.29)	2.37	**0.02**	0.36 (0.31, 0.42)	13.3	**< 0.001**	**$p < 0.001$**, $F_{(4,64)} = 255.9$, $R^2_{adj.} = 0.94$
	Meta	1	–	–	–	0.44 (0.34, 0.49)	1.40	0.17	
	Tad	23	0.50 (0.33, 0.68)	3.84	**< 0.001**	0.28 (0.22, 0.34)	-2.89	**0.005**	
Bufo bufo	Ad	26	0.68 (0.59, 0.76)	16.2	**< 0.001**	0.41 (0.37, 0.44)	25.3	**< 0.001**	**$p < 0.001$**, $F_{(5,66)} = 823.4$, $R^2_{adj.} = 0.98$
	Meta	9	0.48 (0.01, 0.96)	-0.81	0.42	0.36 (0.22, 0.50)	-0.61	0.54	
	Tad	37	1.30 (1.04, 1.56)	4.79	**< 0.001**	0.37 (0.29, 0.46)	-0.79	0.43	
Hyla meridionalis	Ad	25	0.70 (0.60, 0.80)	14.2	**< 0.001**	0.39 (0.36, 0.41)	35.7	**< 0.001**	**$p < 0.001$**, $F_{(5,44)} = 452.2$, $R^2_{adj.} = 0.98$
	Meta	5	0.02 (-1.15, 1.19)	-1.17	0.25	0.29 (0.16, 0.41)	-1.61	0.12	
	Tad	20	0.72 (0.59, 0.86)	0.31	0.76	0.36 (0.32, 0.39)	-1.66	0.10	
Pseudis paradoxa	Ad	22	0.42 (0.19, 0.65)	3.62	**< 0.001**	0.53 (0.43, 0.63)	10.7	**< 0.001**	**$p < 0.001$**, $F_{(5,44)} = 178.1$, $R^2_{adj.} = 0.95$
	Meta	5	0.57 (0.16, 0.97)	0.74	0.46	0.35 (0.15, 0.55)	-1.86	0.07	
	Tad	23	0.66 (0.41, 0.90)	1.96	0.06	0.37 (0.27, 0.47)	-3.24	**0.002**	
Aubria subsigillata	Ad	14	0.85 (0.77, 0.94)	21.2	**< 0.001**	0.44 (0.41, 0.47)	27.6	**< 0.001**	**$p < 0.001$**, $F_{,(4,26)} = 947.5$, $R^2_{adj.} = 0.99$
	Meta	1	–	–	–	0.50 (0.41, 0.59)	1.42	0.17	
	Tad	16	1.99 (1.82, 2.15)	14.2	**< 0.001**	0.39 (0.34, 0.44)	-1.94	0.06	
Polypedates lecuomystax	Ad	21	0.60 (0.25, 0.94)	3.48	**0.001**	0.57 (0.45, 0.69)	9.66	**< 0.001**	**$p < 0.001$**, $F_{(5,54)} = 303.6$, $R^2_{adj.} = 0.96$
	Meta	7	0.73 (-0.63, 2.09)	0.19	0.85	0.43 (0.25, 0.62)	-1.53	0.13	
	Tad	32	1.38 (1.00, 1.77)	4.10	**< 0.001**	0.42 (0.30, 0.55)	-2.32	**0.02**	
Kaloula pulchra	Ad	23	0.67 (0.60, 0.73)	19.5	**< 0.001**	0.39 (0.36, 0.42)	28.2	**< 0.001**	**$p < 0.001$**, $F_{(5,33)} = 431.1$, $R^2_{adj.} = 0.98$
	Meta	7	0.66 (0.22, 1.10)	-0.03	0.97	0.29 (0.17, 0.40)	-1.81	0.08	
	Tad	9	0.92 (0.48, 1.36)	1.17	0.25	0.31 (0.22, 0.39)	-2.12	**0.04**	

Table 2 (continued)

Taxon	Life stage	*N*	*b* (95 % CI)	t_b	p_b	*a* (95 % CI)	t_a	p_a	Model significance
Microhyla rubra	Ad	27	0.71 (0.60, 0.82)	12.9	**< 0.001**	0.35 (0.33, 0.37)	39.3	**< 0.001**	**$p < 0.001$**, $F_{(4,37)} = 139.6$, $R^2_{adj.} = 0.93$
	Meta	1	–	–	–	0.33 (0.23, 0.43)	-0.44	0.66	
	Tad	14	0.31 (0.01, 0.61)	-2.66	**0.01**	0.12 (0.05, 0.20)	-6.18	**< 0.001**	
Hemisus marmoratus	Ad	13	1.30 (0.88, 1.73)	6.16	**< 0.001**	0.15 (0.07, 0.22)	3.90	**< 0.001**	**$p < 0.001$**, $F_{(5,45)} = 59.9$, $R^2_{adj.} = 0.85$
	Meta	14	-0.61 (-1.36, 0.14)	-5.14	**< 0.001**	0.27 (0.18, 0.36)	2.67	**0.01**	
	Tad	24	0.68 (0.19, 1.16)	-2.63	**0.01**	0.22 (0.14, 0.30)	1.71	0.09	
Leptopelis spiritusnoctis	Ad	21	0.31 (0.03, 0.59)	2.23	**0.03**	0.64 (0.58, 0.69)	23.3	**< 0.001**	**$p < 0.001$**, $F_{(3,38)} = 746.8$, $R^2_{adj.} = 0.98$
	Tad	21	1.32 (0.96, 1.68)	5.67	**< 0.001**	0.35 (0.25, 0.45)	-5.93	**< 0.001**	
Trichobatrachus robustus	Ad	26	0.71 (0.58, 0.85)	10.3	**< 0.001**	0.56 (0.51, 0.66)	14.8	**< 0.001**	**$p < 0.001$**, $F_{(5,48)} = 310.4$, $R^2_{adj.} = 0.97$
	Meta	8	0.71 (0.04, 1.39)	0.00	1.00	0.38 (0.21, 0.55)	-2.48	**0.02**	
	Tad	20	0.75 (0.55, 0.94)	0.34	0.74	0.44 (0.36, 0.52)	-3.52	**< 0.001**	

Ad = adults, Meta = metamorphs, Tad = tadpoles. Significant *p* values are in bold

different (and negative) slope in metamorphs, while *T. robustus* metamorphs had a significantly lower intercept than adults (Table 2).

Comparison of tadpole and adult ontogenetic allometries across species

We found that ontogenetic eye-body allometry can vary substantially across species in both the tadpole and the adult stages of biphasic anuran ontogeny. Linear mixed models allowing variable eye-cube root of mass allometric slopes across species fit the data significantly better than those assuming a common slope in both tadpoles ($\Delta AIC = 110$) and in adults ($\Delta AIC = 29$). Variable slope models were also better supported in models of eye-SVL allometry for tadpoles ($\Delta AIC = 110$) and adults ($\Delta AIC = 38$) (Table 3).

Further, SMA regressions of log eye diameter vs. log the cube root of body mass × species indicated a significant effect of species on allometry in both tadpoles ($p < 0.0001$, Lik. ratio stat. = 177.5, df = 11) and adults ($p < 0.0001$, Lik. ratio stat. = 56.3, df = 11). Pairwise comparisons of allometric slopes across species showed that, in general, tadpoles differed more among species than adults did (Fig. 3a, b). Whereas 56 pairwise comparisons were significantly different among tadpole slopes, only 25 pairwise comparisons were significantly different among adult slopes. Furthermore, significantly different slopes among adults were mostly explained by comparisons to two species: *H. marmoratus* and *X. victorianus*. The relatively small eyes of *Hemisus* and *Xenopus* (compared to other anurans; see Thomas et al. 2020), which are highly fossorial and aquatic, respectively, may explain the significant differences between their slopes and those of the other focal species. We note,

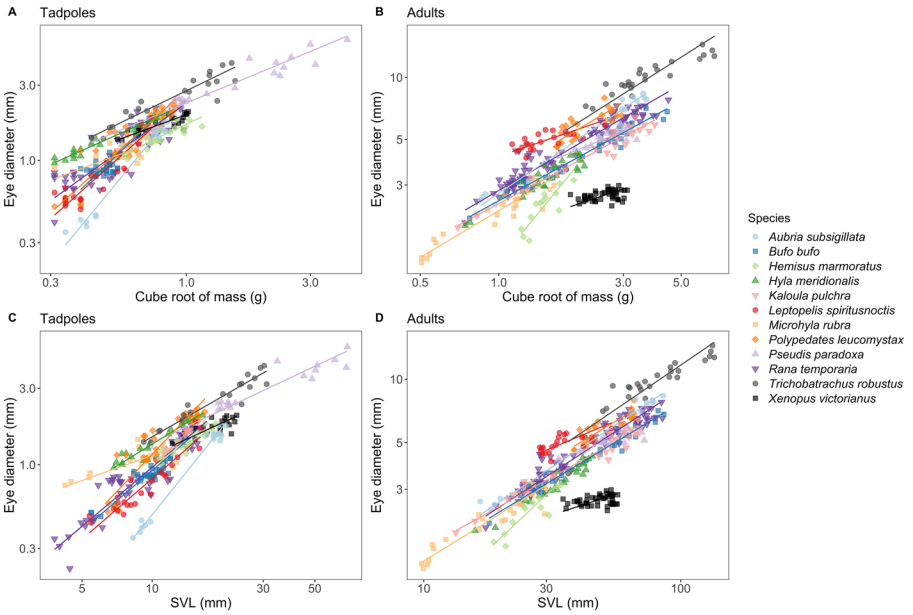

Fig. 3 Eye-body size ontogenetic allometry across major life stages in 12 species of anuran amphibians. Eye-body allometric comparisons for tadpoles (**a**) and adults (**b**) using eye diameter and the cube root of mass are comparable to eye-body allometries where snout-vent length (SVL) is used as a proxy for body size (**c**, **d**). Points and lines are coloured by species, and lines indicate SMA regressions

however, that although *H. marmoratus* had some of the smallest eyes among adults we sampled, this species had a high adult slope (Fig. 3).

Effects of ecology on ontogenetic eye-body allometry

Phylogenetic linear mixed models implemented in MCMCglmm showed significant effects of both tadpole and adult ecology on eye-body ontogenetic allometry in anurans. Tadpole microhabitat, tadpole eye position, tadpole mouth position, adult habitat, and adult activity period all had significant effects on the log-log scaling of eye diameter with the cube root of mass in tadpoles. Species with benthic tadpoles (vs. nektonic), dorsal tadpole eyes (vs. lateral), anteroventral tadpole mouths (vs. ventral or terminal), non-aquatic and non-fossorial adult habitats (vs. fossorial or aquatic), and nocturnal adult activity patterns (vs. both diurnal and nocturnal) had significantly higher slopes for eye-body allometry as tadpoles (Table 4). However, among adults, only tadpole eye position had a significant effect on eye-body allometry. The difference was in the same direction as found in tadpole eyes; species with dorsal eyes as tadpoles had higher slopes for eye-body scaling as adults than species with lateral eyes as tadpoles (Table 4). Comparisons of eye scaling with SVL showed similar ecological effects and trends, though more tadpole traits had effects on adult allometric slopes in these models (Table S9).

Given these results, we looked at mean relative eye sizes across tadpole microhabitats and found no difference between species with benthic or nektonic tadpoles for early (< stage 31) tadpoles (Kruskal–Wallis: $\chi2 = 0.32$, $df = 1$, $p = 0.57$). However, relative eye size in late tadpoles (stages 31–40) and adults (stage 46) are significantly different among benthic and nektonic microhabitats (Kruskal–Wallis, $\chi2 = 4.8$, $df = 1$, $p = 0.03$; and $\chi2 = 6.3$, $df = 1$, $p = 0.01$, respectively).

Discussion

We find evidence of shifts in ontogenetic eye-body allometry at metamorphosis in anurans, with decreased investment in eye growth in adults compared to tadpoles. There is greater variation in allometric relationships among species in tadpoles than in adults, and these differences correlate with tadpole ecology. Based on these findings, we have organised the discussion into three sections where we interpret (1) the putative ontogenetic decoupling of tadpole and adult eye growth trajectories across many of the species we studied, (2) the variable patterns of eye growth observed in metamorphs, and (3) the discovery that multiple ecological factors are significantly associated with variation in tadpole slopes. We compare these findings with our a priori predictions and conclude with future directions for investigating the role of vision during early life stages in species with complex life cycles.

Eye-body allometries of tadpoles can differ from their adult counterparts

With the exception of the Mediterranean tree frog, *H. meridionalis*, anuran eye-body allometries in tadpoles differed from those of their conspecific adults as predicted (Fig. 2; Table 2). This result is consistent with previous studies, which demonstrate that drivers of overall morphological variation in tadpole and adult anurans are largely decoupled (Roelants et al. 2011; Sherratt et al. 2017; Phung et al. 2020). Among eight species that showed a significant difference in slope from tadpole to adult stages, six had higher slopes during

the tadpole stage, consistent with our prediction that higher slopes in tadpoles facilitate large eye sizes in adults. In fishes, this pattern of investment is thought to enable the concomitant transition to visual predation in adults (Gisbert 1999; Saemi-Komsari et al. 2018). In three of our study species, allometry differed only in intercepts among stages, and relative eye growth was the same before and after metamorphosis. This pattern was present in *K. pulchra*, *P. paradoxa*, and *T. robustus* (Table 2; Fig. 2).

The presence of a constant eye scaling slope before and after metamorphosis in four species we sampled is surprising, because anuran tadpoles and adults often have contrasting ecologies. For instance, the banded bullfrog, *K. pulchra*, has nektonic, sometimes diurnally-active aquatic tadpoles, whereas the terrestrial adults are subfossorial, nocturnal, and rarely exposed to daylight (Table 1; J. Streicher, pers. obs.). It is possible that with increased ontogenetic sampling within species we would have the power to detect significant differences between tadpole and adult slopes for additional species. However, it is clear that some anurans (e.g., *H. meridionalis, T. robustus*) maintain a constant rate of eye growth relative to body growth throughout ontogeny, despite drastic changes in morphology, physiology, and ecology at metamorphosis. It may be promising to examine how other aspects of the visual system (e.g., lenses, pupil shape) change at metamorphosis in species with variable allometric patterns of eye growth.

We predicted that across species, tadpoles would have more conserved eye-scaling relationships than adults, because all tadpoles in our study occupy aquatic habitats but the adults exhibit a mixture of (semi)aquatic and terrestrial lifestyles. In contrast to our prediction, tadpole slopes and intercepts varied more than adult slopes and intercepts (Fig. 3; Table 3). This could be due to real differences among species or measurement error because, with few exceptions, tadpoles are smaller than adults. Measurement error, however, seems unlikely to explain the differences, because R^2 values, which should be lowered by substantial measurement error, were not consistently lower in tadpoles than adults (Table S5). More variable allometries of tadpoles may relate to differences in hatching times among species, or may reflect the diverse relative eye sizes found among adults.

Individuals undergoing metamorphosis vary in eye growth trajectory

Metamorphosis is a massive physiological and morphological change for amphibians. Several of the changes that occur when transitioning from an aquatic tadpole to an often terrestrial adult involve the visual system (Hoskins 1990). These changes include modifications to the neurological mechanisms of vision (e.g., *Xenopus laevis*, von Uckermann et al. 2016), development of accessory structures (e.g., *Ansonia*, *Amolops*, and *Scaphiopus*, Nodzenski and Inger 1990; Hall et al. 1997), and changes in lens shape (e.g., *Pelobates syriacus*, Sivak and Warburg 1983). Given the concomitant and dramatic changes to the visual system and body plan that occur during this period, the allometry of metamorphs is particularly interesting to consider. We found that across species, some metamorphs had relative eye-sizes that fell along the tadpole eye allometry trajectory, while others were more adult-like, and some appeared split between life stages (Fig. 2; Table 2). Further, when any allometric shift was detected in a species, tadpoles and adults always exhibited significant differences in eye scaling; patterns were not driven by metamorphs. This, and the absence of any allometric difference between juveniles/subadults and mature adults in *Rana temporaria* (Fig. 1), suggests that metamorphosis is the key event driving the rapid shift in eye-body ontogenetic allometry observed among some anuran species.

In species that exhibit change in allometric slope, the relative investment in eye growth clearly changes. In species that exhibit only a shift in intercept, however, allometric shifts may be driven entirely by changes in body size at metamorphosis unrelated to eye growth. For example, the paradoxical frog, *Pseudis paradoxa*, has a unique life history in which the nektonic tadpoles can be larger than adults (Downie et al. 2009). The rapid decrease in body mass during metamorphosis results in larger relative eye sizes among adults and can explain the increased intercept for adult eye scaling compared to tadpoles and metamorphs (Fig. 2e).

Ecology is associated with rapid larval eye growth

We discovered that tadpoles tend to exhibit a higher slope for eye-body scaling than conspecific adults, though this trend was not universal across species we examined, and tadpole allometry varied substantially among species (Figs. 2 and 3; Table 2). Why do some species "invest early" in their eyes? Prioritising eye growth early in development may reflect tadpole reliance on vision for obtaining food and/or avoiding predation, but could also be necessary to produce large relative eye sizes in highly visual adults. Our results suggest that variation in tadpole slopes results from a combination of both tadpole and adult visual ecology.

Anuran larval ecology is known to influence several aspects of tadpole morphology (Altig and Johnston 1989; Nodzenski and Inger 1990; Sherratt et al. 2018), and, in some groups, adult body size (Phung et al. 2020). We observed a significant association between tadpole microhabitat (benthic versus nektonic) and tadpole eye-body allometric slope (Fig. 4a; Table 4). On average, species with benthic larvae had significantly higher tadpole slopes, indicating faster relative eye growth during larval periods. There was also a significant association between tadpole slope and eye position (dorsal versus lateral, Fig. 4b; Table 4), and mouth position (anteroventral versus others; Table 4), and these relationships are also likely explained by tadpole microhabitat. Anuran species with benthic tadpole ecologies typically have dorsal eyes (regardless of whether they occur in lentic or lotic habitats) and lateral eyes are most common in lentic nektonic tadpoles (Altig and McDiarmid 1999). Similarly, anteroventral mouths are often observed in lentic, benthic tadpoles (Altig and Johnston 1989), and this common pairing of traits occurs in six (out of eight) benthic species in our study (Table 1).

In spite of these strong associations, a scenario where benthic tadpoles require enhanced vision is not supported by either absolute eye sizes or feeding behaviours. First, tadpoles with benthic ecologies generally have smaller absolute eye sizes than nektonic forms (Thibaudeau and Altig 2012); a pattern supported by our dataset where the average absolute eye size of benthic tadpoles was 1.25 mm ($N=206$, $SE=0.05$) versus 1.82 mm ($N=96$, $SE=0.10$) for nektonic tadpoles. Second, although nektonic tadpoles forage in the water column where vision facilitates the location of prey, benthic tadpoles are mostly grazers feeding generally on sessile biofilms and only occasionally consume microscopic animals (Altig and Johnston 1989). Thus, benthic tadpoles likely depend on olfaction more than vision for locating their primary food sources (e.g., Veeranagoudar et al. 2004). By contrast, predator avoidance may support a scenario where benthic tadpoles require larger relative eye sizes than their nektonic counterparts. On average, benthic tadpoles have smaller body sizes and slower rates of feeding than nektonic larvae (Venesky et al. 2013), increasing their susceptibility to predation (Richards and Bull 1990). They also typically have greater exposure to predators and mortality than their nektonic counterparts (Peterson

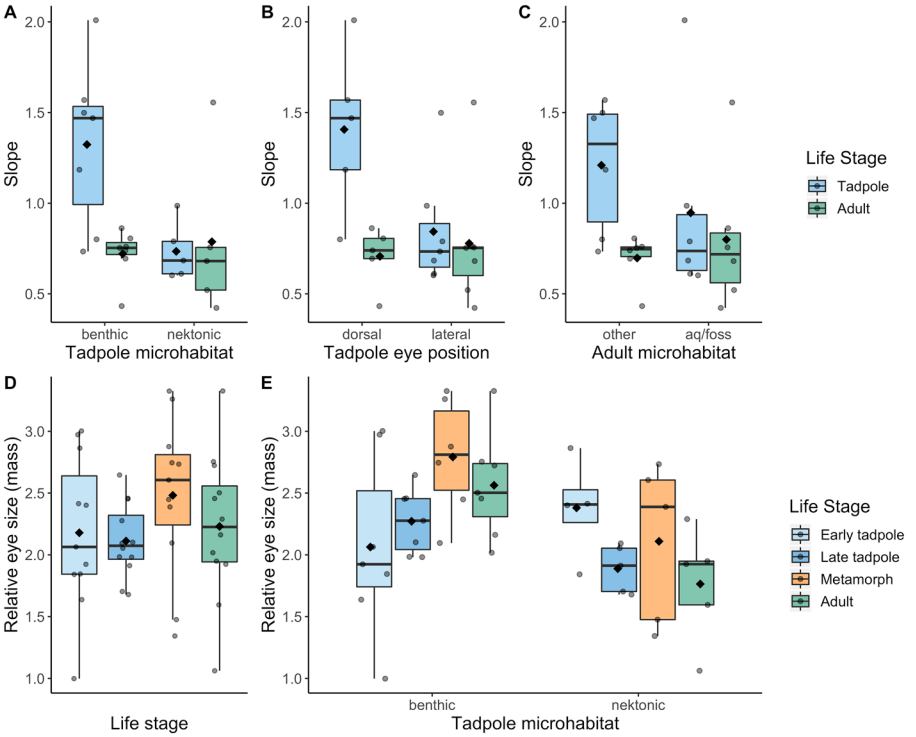

Fig. 4 Tadpole and adult slopes from OLS regressions of eye-body mass allometry in each species compared to tadpole microhabitats (benthic versus nektonic; **a**), tadpole eye positions (dorsal versus lateral; **b**), and adult habitats (aquatic/fossorial vs. other; **c**). Mean relative eye sizes (eye diameter [mm]/cube root of mass [g]) across all species for each major life stage with tadpoles split into early (<Gosner stage 31) and late (Gosner stage 31–40) groups (**d**) and for major life stages separated by benthic and nektonic species (**e**)

Table 3 Comparison of two linear mixed models of ontogenetic eye-body allometry fitted with restricted maximum likelihood (REML) using Akaike's information criterion (AIC)

Group	Measure of body size	Model type	AIC	ΔAIC
Tadpoles	Cube root of mass	Variable intercepts	− 559	0
		Variable slopes	− 669	110
	SVL	Variable intercepts	− 579	0
		Variable slopes	− 689	110
Adults	Cube root of mass	Variable intercepts	− 1129	0
		Variable slopes	− 1158	29
	SVL	Variable intercepts	− 1179	0
		Variable slopes	− 1217	38

One model allowed variable intercepts but used a common allometric slope among species groups, and the other allowed variable slopes and intercepts across species. Models included $\log_{10}$ eye diameter as the response variable, $\log_{10}$ body size (SVL or the cube root of mass) as a fixed effect, and species identity as a random effect, and were applied separately to tadpoles and adults

Table 4 Results of phylogenetic linear mixed models where the response variable was $\log_{10}$ eye diameter, fixed effects were $\log_{10}$ cube root of body mass and an ecological trait, and random effects were species identity and the phylogenetic non-independence among species

Life stage	Ecology fixed effect	State	Slope (95% HPD)	Eff. sample	pMCMC	Intercept (95% int)	Eff. sample	pMCMC
Tadpoles	Tadpole microhabitat	Benthic	1.11 (1.03, 1.19)	4995	**0.0002**	0.35 (0.22, 0.47)	4995	**0.0008**
		Nektonic	0.63 (0.49, 0.76)	4995	**0.0002**	0.27 (0.09, 0.43)	4995	0.33
	Tadpole eye position	Dorsal eyes	1.12 (1.02, 1.22)	5019	**< 0.0001**	0.32 (0.18, 0.45)	4995	**0.0004**
		Lateral eyes	0.80 (0.67, 0.93)	4995	**< 0.0001**	0.30 (0.13, 0.46)	5255	0.74
	Tadpole mouth position	Anteroventral	1.08 (1.02, 1.15)	4995	**< 0.0001**	− 1.11 (− 1.25, −0.97)	4995	**< 0.0001**
		Terminal	0.44 (0.22, 0.65)	5331	**< 0.0001**	− 0.35 (− 0.67, −0.03)	4995	**< 0.0001**
		Ventral	0.80 (0.60, 1.01)	4995	**0.008**	− 0.61 (− 1.03, −0.22)	4995	**0.013**
	Adult habitat	aq/foss	0.83 (0.73, 0.94)	5375	**0.0002**	0.26 (0.13, 0.38)	4995	**0.0002**
		Other	1.02 (0.89, 1.17)	5283	**0.007**	0.35 (0.19, 0.51)	4995	0.29
	Adult activity period	Both	0.78 (0.70, 0.87)	4995	**0.0002**	0.30 (0.14, 0.44)	4995	**0.002**
		Nocturnal	1.15 (1.02, 1.29)	4995	**0.0002**	0.34 (0.16, 0.50)	4995	0.63
Adults	Tadpole microhabitat	Benthic	0.71 (0.68, 0.75)	4995	**0.0002**	0.48 (0.35, 0.60)	4995	**0.0002**
		Nektonic	0.65 (0.58, 0.72)	4995	0.06	0.32 (0.15, 0.50)	4995	0.08
	Tadpole eye position	Dorsal eyes	0.72 (0.68, 0.75)	4493	**< 0.0001**	0.48 (0.32, 0.64)	4995	**0.0004**
		Lateral eyes	0.65 (0.59, 0.71)	4995	**0.0496**	0.60 (0.17, 0.56)	5322	0.22
	Tadpole mouth position	Anteroventral	0.71 (0.68, 0.74)	4753	**< 0.0001**	0.43 (0.30, 0.57)	4374	**< 0.0001**
		Terminal	0.64 (0.58, 0.72)	4995	0.08	0.30 (0.05, 0.53)	4995	0.24
		Ventral	0.72 (0.60, 0.83)	4995	0.92	0.57 (0.21, 0.90)	4995	0.39
	Adult habitat	aq/foss	0.71 (0.66, 0.76)	4995	**0.0002**	0.33 (0.19, 0.47)	5301	**0.0004**
		Other	0.69 (0.63, 0.75)	4995	0.47	0.48 (0.30, 0.67)	4995	0.10
	Adult activity period	Both	0.71 (0.66, 0.75)	5073	**0.0002**	0.37 (0.16, 0.56)	5014	**0.003**
		Nocturnal	0.69 (0.63, 0.46)	5566	0.58	0.43 (0.19, 0.65)	4995	0.57

Parameter estimates, the 95% highest posterior density (HPD), and MCMC '*p* values' (pMCMC) are listed (bolded when significant); pMCMC for the first state listed for each fixed effect indicates whether coefficients differ significantly from 0. *p* values for other states indicate the significance of the contrast with the first listed state

et al. 1992; Phuge and Phuge 2019), and their upward-facing eyes are oriented to detect potential predators above.

Another possible explanation for the association between larval ecology and allometric slopes relates to adult ecology. Adult relative eye size also differed among species with benthic and nektonic larvae (Fig. 4e), and adult visual needs may drive higher allometric eye scaling slopes in benthic tadpoles. We found that benthic and nektonic tadpoles early in development have similarly sized eyes, but relative eye size increases through ontogeny in benthic tadpoles (which in our sampling tend to have terrestrial adults with large eyes) and decreases in nektonic tadpoles (which tend to have aquatic or fossorial adults with smaller eyes) (Fig. 4e). Additionally, species occupying habitats associated with small relative eye sizes as adults (those with aquatic, fossorial, and subfossorial ecologies; Thomas et al. 2020) had significantly lower allometric slopes as tadpoles than those occupying semiaquatic, ground-dwelling, and scansorial habitats as adults. Further, *M. rubra* and *H. marmoratus*, both fossorial species with small adult eyes, were the only two species with significantly lower slopes during the tadpole stages than as adults. This is consistent with faster eye growth rates in the tadpoles of species that have proportionally larger eyes as adults (Fig. 4c; Table 4). We also observed that another aspect of adult ecology, activity pattern, predicted differences in tadpole slopes, with nocturnal species having higher tadpole slopes (Table 4), as is expected given that nocturnal species have, on average, proportionally larger eyes as adults (Thomas et al. 2020).

Intriguingly, only one of the ecological characteristics we tested was significantly associated with variation in adult slopes (Table 4). We found that species with lateral eyes as tadpoles had lower adult slopes than their counterparts with dorsal eyes as tadpoles; however, the confidence intervals in adult slope estimates overlapped substantially between eye placement categories and the difference was much smaller than differences observed among larval slopes (Table 4). Fully understanding the effect of adult eye size on tadpole eye scaling and disentangling the contributions of larval vs. adult ecology in driving anuran ontogenetic eye-body allometry will require further sampling across species with diverse ecologies and visual needs.

Conclusions and future directions

In adult anurans, investment in relative eye size is among the highest of all major vertebrate groups (Thomas et al. 2020), suggesting a major role for vision in the behaviours of most species. Vision is also critical during the tadpole stage for phototaxis (Blackiston and Levin 2013), conspecific recognition (Rot-Nikcevic et al. 2006; Brett Sutherland et al. 2009), and predator avoidance (Hettyey et al. 2012). Our study provides further evidence for the important role of vision during biphasic anuran lifecycles, and suggests that the visual ecology of tadpoles, and potentially their corresponding visual ecologies as adults, contribute to interspecific differences in relative larval eye growth.

Future research on ontogenetic eye-body allometry in a broader sampling of amphibians would greatly aid in interpreting the results we present here. First, more comprehensive sampling of species with differing ecologies (both tadpole and adult) would allow for more robust statistical tests of the putative ecological associations we found. Second, dense sampling of metamorph individuals would aid with interpreting patterns of changes at the crossroads of metamorphosis. Finally, extensive phylogenetic sampling would also be helpful to test for generality in other aspects of visual system development during a biphasic lifecycle, including changes in lens shape and the development of accessory structures.

Appendix

Specimens examined. Unless otherwise noted all measurements were taken by S. Shrimpton. BMNH = The Natural History Museum, London; ZMB = Museum für Naturkunde, Berlin. *Measured by K. Thomas.

Arthroleptidae, *Leptopelis spiritusnoctis* (N = 44).

ZMB 81,106 (1–11), 79,630 (1–4), 79,633 (1–7), 79,635, 79,631, 88963–67, 79,825, 86,034, 81,086, 78,581, 79833–34, 79830–31, 79828–29, 77891–92, 81,082, 81,094, 81,102.

Arthroleptidae, *Trichobatrachus robustus* (N = 55).

BMNH 1907.5.22.51*, 1969.486*, 1949.1.3.51*, 1936.3.4.97–98*, 1982.745*, 1936.3.4.95–96*, 1969.841*, 1906.5.28.23*, 1969.483–85*, 1969.482, 1958.1.4.76–78*, 1969.1592–93*, 1936.3.4.103–105*, 1936.3.4.107*, 1936.3.4.101–02*, 1958.14.71–73, 1958.14.75–77, 1904.7.1.19–20, 1904.7.1.11–13, 1980.1432.

ZMB 82,033, 82,042, MH0438, 82,039 (1–6), 82,050 (1–2), 82,047 (1–3) 82,043 (1–2), 82,034.

Bufonidae, *Bufo bufo* (N = 75).

BMNH 1961.973–81, 1973.758, 1951.1.4.62, 1893.8.22.2, 1973.759–60, 1894.7.20.3–4, 1968.887–88, 1886.1.22 (11–25), 1934.10.18.1, 1936.12.3.18, 1964.320–22, 1954.1.3.91–94, 1954.1.3.79–91, 1964.646–47, 1949.1.8.49–50.

ZMB 34.421–34,501 (1–6), 30,775 (1–9), 87,747.

Hemisotidae, *Hemisus marmoratus* (N = 51).

BMNH 2005.999–1014, 2005.1201, 2002.211–14, 1986.1266 (1–2), 1986 – 1264, 2005.1301.

ZMB 11.5 (2), 7.5.153.1, 7.5 (1–3), 20.6.153, 23.6.93, 11.8.152 (1–2), 79,847, 80,193, NA-Pond-Sample-1–15.

Hylidae, *Hyla meridionalis* (N = 50).

BMNH 1913.8.30.10, 1947.1.3.96, 1984.11.20.114–16, 1920.1.20.3806 (1–14), 1890.1.22 (4–11), 1928.12.20.176–94, 1920.1.20.1943 (1–4).

Hylidae, *Pseudis paradoxa* (N = 52).

BMNH 1971.1632–33, 1856.5.14.5, 1866.8.14.257, 1987.2431, 1894.3.14.107–14, 1976.142–47, 1909.4.30.22–24, 1927.8.1.43, 1937.7.29.11, 1937.7.29.23, 1946.4.2.58 (239.A), 1946.4.2.59 (240.A), 1946.4.2.60 (258.A), 1946.4.2.61 (237.A), 1946.4.2.62 (223A), 1937.7.29.14, 1892.6.21.9, 1894.3.14 (116 – 18), 1946.4.2.68–70, 1927.8.1.42, 1978.1330 (1–12).

ZMB 13,936, 3191.

Microhylidae, *Kaloula pulchra* (N = 39).

BMNH 1959.15.40–41, 1898.11.8.73–75, 1898.11.8.76A–B, 1973.890–96, 1974.3228, 1974.3232, 1898.11.8.71–72, 1928.12.13.131–43, 1859.7.1.30 (1–2), 1893.9.6.1, 1974.3235 (1–3), 1896.6.25.90–91.

Microhylidae, *Microhyla rubra* (N = 45).

BMNH 1955.1.10.67, 1895.12.30.50, 1876.3.21.48, 1908.7.2.12.13 (1–2), 1955.1.10.66, 1973.3006–7, 1872.4.17.233–34, 1982.1286–89, 1846.11.21.60–66, 1872.1.26.32 (1–3), 1903.9.26.29–33, 1972.1894 (1–2), 1955.1.10.68–75, 1955.1.10.61–64, 1874.4.29.268, 1887.2.26.24.

Pipidae, *Xenopus victorianus* (N = 69).

BMNH 1977.1429–73, 1977.1506–29.

Pyxicephalidae, *Aubria subsigillata* (N = 31).

BMNH 1980.1375 (1–6), NA-27.6.17 (1–10).

ZMB 88,433, 84715–20, 79,260, 71,279, 30,997, 84724–26, 71,278, 83,453.

Ranidae, *Rana temporaria* (N = 152).

BMNH 1966.331–33, 1988.177.921, 1970.758–62, 1956.1.8.9–12, 1969.2844 (1–6), 1983.921, 1968.941, 1964.645, 1933.3.7.11, 1982.1315–16, 1886.1.22 (5–19), 1896.4.18 (1–2), 1933.3.7.3, 1933.3.7.6, 1933.3.7.8–9, 1897.12.31.7–9, 1897.12.31.11–12, 1894.2.26.14–15, 1956.1.8 (13–18), 1969.2843 (1–15), 1932.9.3.1, 1963.896–900, 1881.5.9 (1–4), 1968.936–37, 1920.1.20.2958A–D, 1903.730.1–2, 1891.11.7.1–6, 1900.9.11.2, 1949.1.2.99, 1972.1699–1711, 1887.8.25 (14–17), 1965.824, 1972.1684–98, 1895.9.7.32–36. 1894.5.7.25–29, 1920.1.20.216 (1–9).

Rhacophoridae, *Polypedates leucomystax* (*N* = 60).

BMNH 1896.6.25.140–44, 1896.6.25.130–37, 1896.6.25.120–24, 1974.3694, 1974.3690, 1974.3692, 1974.3688, 1974.3693, 1974.3689, 1973.1344–48, 1974.4825–27, 1974.4832, 1974.4829, 1967.2595, 1967.2609–10, 1967.2590–91, 1896.2.29.149–154, 1974.4834 (1–7).

ZMB 33.809 (1–7), 64,507.

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Code availability Annotated code to reproduce all analyses and quantitative aspects of figures are available on the following GitHub repository: https://github.com/knthomas/anuran-allometry.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethics approval All animal material examined in this study came from existing museum collections.

Consent to participate Not applicable.

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