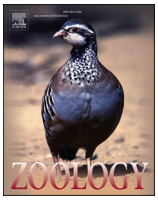
Zoology 152 (2022) 126011

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.com/locate/zool



Latent effect of larval rearing environment on post-metamorphic brain growth in an anuran amphibian

Hanna Peters, Frédéric Laberge *, Andreas Heyland

Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph N1G 2W1, ON, Canada

ARTICLE INFO

Keywords:
Amphibians
Visual restriction
Developmental rate
Metamorphosis
Brain size

ABSTRACT

Early development is highly susceptible to environmental influence. We evaluated the role of larval visual environment on brain morphology plasticity in late larval and juvenile stages of *Bombina orientalis*, an anuran amphibian changing from an aquatic to a terrestrial habitat after metamorphosis. Manipulation of the visual environment was achieved by rearing larvae in normal and darkened water. The juveniles were exposed to normal lighting conditions after metamorphosis, allowing to assess if plastic effects persisted or emerged after metamorphosis. The darkness treatment accelerated development before slowing it down substantially, allowing controls to metamorphose earlier. Although larvae reared in darkened water had the same relative brain size as controls by the end of the larval period, juveniles that had been reared in darkened water as larvae had brains that were 14.4% smaller than juveniles that had been reared under control conditions. Conversely, relative telencephalon size was 6.7% larger in juveniles previously reared in darkened water compared with controls, again with no effect of darkened water seen by the end of the larval period. Unlike the latent effects seen on whole brain and telencephalon size, relative size of the optic tectum was significantly smaller in both larvae and juveniles exposed to the darkened water treatment. Therefore, the effects of visual restriction on juvenile brain form were a combination of latent (whole brain and telencephalon) and carry-over (optic tectum) developmental effects.

1. Introduction

Conditions experienced during development can impact the phenotype of organisms in later stages of life, with variable effects on fitness. For example, chemical exposure during development can interfere with adult behavior and reproduction in aquatic wildlife, e.g. (invertebrates: Lewis and Ford, 2012), (fish: Frank et al., 2019; Porseryd et al., 2019), (amphibians: Rohr and Palmer, 2005; Hayes et al., 2011), and a variety of perinatal stressors are believed capable of altering human developmental trajectories, resulting in adult pathologies (Padmanabhan et al., 2016). Conversely, developmental programming of offspring to anticipate future conditions can result in fitness benefits in variable environments (Strathmann et al., 1993; Hart and Strathmann, 1994; Miner and Vonesh, 2004; Dantzer et al., 2013; Sheriff and Love, 2013).

In addition to the negative or positive aspects of developmental effects, variation in the onset and offset of phenotypic effects following controlled exposure has been noted. Many invertebrate phyla and amphibians undergo an indirect developmental trajectory characterized by a drastic metamorphosis between the larval and juvenile stages (Moran, 1994; Hall and Wake, 1999; Bishop et al., 2006; Heyland and Moroz, 2006). How larval experience translates into phenotypic and molecular changes post-metamorphosis has received some attention. Specifically, physiological, morphological or developmental changes during the larval stage brought about by experimental manipulations may have little impact on the juvenile or adult form, presumably due to the drastically different environments of these life history stages and associated divergent selection pressures (e.g. Moran, 1994; Campero et al., 2008; Marshall and Morgan, 2011; Woodley et al., 2015). Still, larval changes have been shown to persist after metamorphosis as developmental carry-over effects; a type of effect that has been well documented and can have major consequences for post-metamorphic performance (e.g. Pechenik et al., 1998; Relya, 2007; Crean et al., 2011; Fadl et al., 2019; Bredeweg et al., 2019). Alternatively, there are examples of latent effects, where changes do not manifest during the larval period, but emerge at a later stage after metamorphosis (Pechenik, 2006). Clear linkages between experimental manipulations and phenotype are not limited to events separated by metamorphosis, as the effects of embryonic exposure can already manifest after hatching in anuran larvae

* Corresponding author.
E-mail addresses: peters.hanna@outlook.com (H. Peters), flaberge@uoguelph.ca (F. Laberge), aheyland@uoguelph.ca (A. Heyland).

https://doi.org/10.1016/j.zool.2022.126011
Received 15 October 2021; Received in revised form 12 March 2022; Accepted 18 March 2022
Available online 24 March 2022
0944-2006/© 2022 Elsevier GmbH. All rights reserved.

(Garcia et al., 2017). In the context of developmental plasticity, developmental effects of environmental change are not only critical to understand physiological responses but also to assess the fitness consequences of exposures. Terrestrial anuran amphibians transitioning between aquatic and terrestrial environments in larval and adult stages are an excellent group of organisms to study the timing, mechanisms and consequences of developmentally induced phenotypes and their carry-over and latent effects on post-metamorphic stages.

Environmentally induced plasticity of brain morphology has been reported in larval anurans (Woodley et al., 2015; Gonda et al., 2010; Trokovic et al., 2011) and this form of plasticity can have consequences on behavior. For example, larvae of the common frog *Rana temporaria* grown at high density for 24 days developed a larger optic tectum than larvae grown at low density (Gonda et al., 2010), and this enlargement of the tectum was maintained until shortly before the completion of metamorphosis (Gosner stages 43–44, Trokovic et al., 2011). The enlarged tectum grown at high larval density could be used by larvae to assess the increased complexity of the visual social environment, but it is unclear if this change would persist beyond metamorphosis and influence behavior in older juveniles. Such changes in brain form are thought to be adaptive because observed patterns of brain morphology variation among species reflect ecological specializations (Taylor et al., 1995; Liao et al., 2015; Manzano et al., 2017) and investment in brain tissue comes at a high energetic cost (Niven and Laughlin, 2008).

We were inspired to evaluate the role of the larval visual environment on anuran brain morphology plasticity by the recent study of Pike et al. (2018) who housed wild-caught juvenile nine-spined sticklebacks in darkened water for about 6 months to obtain fish grown in a visually restricted environment. These fish, along with controls grown in normal water, were then tested for their ability to estimate conspecific shoal size using visual or chemical information. Nine-spined sticklebacks show a clear preference for larger shoals of their conspecifics (Coolen et al., 2005). Following testing, fish brains were measured and showed that the visual restriction treatment resulted in plastic changes in brain morphology reducing size of the main visual center and increasing size of the main olfactory center compared to fish reared in normal water (Pike et al., 2018). Notably, behavioral testing showed that brain morphology determined the preferred sensory modality (visual or chemosensory) used by fish to estimate shoal size. Since vertebrates share a basic plan of brain organization and developmental plasticity, we expected that the anuran brain would show changes in sensory brain regions equivalent to those seen in sticklebacks after rearing aquatic larvae in darkened water, with a decrease in optic tectum size (main visual center) and an increase in telencephalon size (main chemosensory brain region). We conducted an experiment using the fire-bellied toad *Bombina orientalis*, an anuran that transitions between aquatic larval and terrestrial post-metamorphic stages, which allowed control of visual restriction until metamorphosis. By assessing brain morphology both at a late larval stage and in juveniles exposed to a normal light environment after metamorphosis, we also assessed if the predicted changes in larval brain morphology would vanish or persist after metamorphosis. Additionally, in the absence of predicted changes in sensory brain morphology by the end of the larval period, the possibility of latent effects on juvenile brain growth could also be investigated.

2. Materials and methods

2.1. Animals

Two groups of six adult *Bombina orientalis* were bred in the Hagen Aqualab facility at the University of Guelph. They were maintained on a diurnal 12:12 h light:dark cycle at a constant temperature of 21 °C. Mating behavior was induced with an initial intra-lymphatic injection of 0.05 μg luteinizing hormone-releasing hormone analog (LHRHa; Millipore-Sigma L4513) dissolved in 100 μl of amphibian saline in each toad, followed within 3 days by a similar injection of 0.5 μg LHRHa. Eggs were usually laid in the morning after the second injection, when they were collected, counted and transferred to plastic tubs containing two liters of aged well water maintained at a constant temperature of 25 °C. The use of group breeding did not allow the assessment of individual contributions to the embryos that were harvested. Larvae hatched about 4–5 days after the eggs were laid. Well water used to raise larvae was replaced at least every third day until they reached stage 25, at which point containers required daily cleaning and water changes. Every second day, larval toads were provided with an amount of boiled lettuce proportional to their size for food. The amount of food was in excess of what the larvae could consume in two days. All procedures were approved by the University of Guelph animal care committee (AUPs #3538 and 3597) under the guidelines of the Canadian Council on Animal Care.

2.2. Developmental atlas

Although *B. orientalis* has previously been used to study environmental effects on development (e.g. Park et al., 2014), no developmental atlas documenting all stages from fertilization to metamorphosis exists for this species. The early developmental stages (up to stage 25) were described by Sussman and Betz (1978). Therefore, we produced an atlas covering all stages from embryogenesis to metamorphosis in *B. orientalis* to provide us with the framework necessary to evaluate potential differences in the rate of development following manipulation of the larval visual environment (Table 1). To aid with staging, the Gosner (1960) simplified staging scheme for anurans was used with additional references to the developmental atlases of Abraham et al. (2018), Nieuwkoop and Faber (1956), Shumway (1940), and Sussman and Betz (1978).

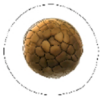
The first batch of eggs obtained from breeding a group of six adults was used for observation of embryonic and larval developmental stages. Protective jelly membranes were removed for a better view of selected embryos. Because amphibian embryos at early stages of development may require the protection offered by jelly membranes (Salthe, 1963; Sive et al., 2007), the embryos selected for observations prior to neurulation were housed in filtered well water until hatching for additional safety. Embryos and larvae were photographed using a camera (model 18MP, AmScope, Irvine, CA) attached to an AZ100 microscope (Nikon). Every 1.5 weeks and at major developmental stages between this interval, 4–5 randomly selected larvae were sacrificed with an overdose of MS-222 and preserved in 10% buffered formalin for characterization of larval stages.

2.3. Manipulation of larval visual environment

A second batch of eggs obtained from breeding the second group of six adults was used to run an experiment. About 130 eggs were put into a control container of well water and an equivalent number of eggs into a container of darkened well water. Darkened water was produced using 0.16 g/L of black food dye (Brilliant Black BN, Millipore-Sigma), following the method of Pike et al. (2018). During the experiment, all manipulations and cleaning were done in darkness with the aid of red-light illumination to minimize light exposure of dark-reared larvae. To reduce density and mitigate potential density-dependent growth inhibition observed in larval amphibians (Girish and Saidapur, 2003), larvae were separated based on size into two different containers for each treatment around stage 25–30 of development. Larval size was equalized one more time around stage 40 by redistribution of larvae between the two tanks of each treatment. We consider potential tank effects unrelated to size differences in later stages of development unlikely because all tanks were submitted to the same conditions, with water changed daily from a common source of aged water and all tanks sharing a common location on a lab bench in the same room.

Larval growth was assessed during nightly water changes at 12, 16 and 25 days of life by measuring snout-vent length (SVL) with a ruler and scoring developmental stage of each individual. In addition,

Table 1
Select stages in embryonic and larval development in the fire-bellied toad, *Bombina orientalis*. Each table row has Gosner stage number on the left, a representative illustration of embryonic/larval morphology and approximate age since fertilization to the right. Additionally, the title column names a stage or a range of stages and a short description of important developmental features and events is provided in the rightmost column. Scale bar represents 1 mm for stages 3–20 and 1 cm for stages 25–46. Illustrations for embryonic stages (stages 3–13) show embryos without their jelly layers where only the perivitelline membrane remains visible.

Stage	Stage Illustration	~Age	Title	Description
3		5–10 hrs	First Cleavage: 2 cell	Embryonic and pre-feeding stages. Embryo undergoes cleavage, gastrulation and neurulation within a protective perivitelline membrane and several jelly layers. The embryo including the capsule is typically 3.5–4 mm in diameter.
4			4 cell	
6		10–24 hrs	16 cell	
7			32 cell	
13			Neurula	
20		70–96 hrs	Gill Circulation	Muscular responses have begun, external gills form from gill buds. Adhesive glands are present ventrally.
25		7 days	Spiracle Appearance	Mouthparts obvious. External gills atrophied. Single medial ventral spiracle forms.
26		8–9 days	Hind Limb Bud Initiation	The limb bud grows until stage 31 followed by incremental indentation to differentiate the toes until stage 42. Pigmentary patterns become increasingly defined.
31		22–25 days	Toe Differentiation and Foot Paddle Development	
41		25–30 days	Forelimb Growth and Emergence	Forelimbs develop within the branchial chamber enclosed laterally by the operculum, often emerging asynchronously at stage 42.
42				
43		30–36 days	Tail Atrophy	Mouth opening ends between nostrils and eyes. Tail atrophies and larva begins resting on terrestrial surfaces.
46		38–42 days	Meta-morphosis	Mouth opening ends posterior to the eyes. Tail is resorbed. Forelimbs increase in strength and juvenile begins predatory terrestrial feeding. Pigmentary patterns are mostly defined. Skin colours are brown, gray and black.

(*continued on next page*)

Table 1 (*continued*)

Stage	Stage Illustration	~Age	Title	Description
				

developmental stage was scored at 22 days of life. Stages of limb bud development (26−30) could not be accurately scored on live larvae, so individuals at this stage of development were recorded as having reached at least stage 26. Survival was assessed during recording periods and container cleaning. As the larvae reached stage 42 (emergence of front limbs), half of these individuals in each container were sacrificed by an overdose of MS-222 and preserved in 10% buffered formalin for later brain measurements (see below). Remaining stage 42 larvae were moved to slightly slanted 2-L plastic boxes with covered lids set up as semi-terrestrial environments by adding gravel at one end and a pool of well water at the other. Air-breathing juveniles moved to the terrestrial side of the box after metamorphosis. To maintain darkness until metamorphosis of the late stage larvae, newspaper was used to cover the semi-terrestrial containers from the darkened water treatment until complete larval tail regression. Thereafter, juvenile toads were exposed to normal light and were kept for an additional 7–10 days after metamorphosis before being sacrificed as described above to study post-metamorphic brain morphology. During this period, juveniles were fed daily with recently hatched pinhead crickets.

2.4. Data analysis

2.4.1. Developmental rate and survival

Larval survival was assessed by comparing survival probabilities between the darkened water treatment and control using a log-rank analysis (Mantel-Cox). We also analyzed age (days post-fertilization) and body size (SVL) in response to the darkened water treatment. Based on our developmental atlas we chose three critical developmental periods for this analysis: stages 30–31, 40–41 and 42–43 (see Table 1 for stage details). We used separate general linear models (GLM) with age or size as dependent variables. Each model had treatment and developmental stage as factors. First, GLM was used to assess the effects of treatment and stage including all developmental stages. Upon statistical significance of the treatment effect, this was followed by separate models of the effect of treatment at each developmental stage to establish patterns of divergence in developmental trajectories. A similar GLM was used to analyze body size including juveniles to assess the potential latent effect of the darkened water treatment on body growth. In this model, SVL of stage 42 larvae and stage 46 juveniles was obtained from the fixed specimens used for brain measurements (see below).

2.4.2. Brain size

Fixed pre-metamorphic larval (stages 42 or 43) and post-metamorphic juvenile (stage 46) specimens were dissected to expose the dorsal surface of the brain. Brains were photographed using the same setup used to photograph embryos and larvae. Three measurements were made from each brain picture (Fig. 1): 1) total dorsal surface area of the brain excluding the medulla oblongata, 2) surface area of both halves of the optic tectum, and 3) surface area of both halves of the telencephalon, including the olfactory bulbs. The polygon selection tool in ImageJ (v1.51j8) was used to obtain area measurements. Measurements were calibrated using photographs of a stage micrometer slide at each magnification used to take brain photographs. Measurement reliability was assessed using intra-class correlation coefficients (ICC) based on repeated measurements of the same set of brain photographs by two lab members (HP and FL). FL was blinded to animal identity before measurements using dummy codes for micrographs. Average ICC and 95% confidence intervals for total brain area (0.99 [0.986–0.995]), optic tectum area (0.92 [0.87–0.95]) and telencephalon area (0.96 [0.86–0.98]) indicated good to excellent reliability (McGraw and Wong, 1996). All brains were subsequently dissected out of the head to obtain measurements of brain, tectum and telencephalon height based on photographs in a lateral orientation. Volume estimates were obtained by multiplying surface area and height. Pearson correlation tests showed very strong correlations between surface area and volume measurements (Brain: $r(43) = 0.94$, $p < 0.001$; Optic Tectum: $r(45) = 0.92$, $p < 0.001$; Telencephalon: $r(44) = 0.96$, $p < 0.001$). We chose to use dorsal surface area to estimate brain and region sizes because the shape of larval toad brains makes height measurements less reliable and specimens with dissection damage to the ventral part of the brain could not be included in volumetric analyses.

The brain size data did not meet parametric statistic assumptions, so generalized linear models (GLMM) were used for the analyses. Size of the whole brain was estimated from its dorsal surface area. Relative brain size was analyzed by using body size (SVL measured in fixed specimens before dissection) as a covariate. Similarly, the size of the optic tectum and telencephalon were estimated by their dorsal surface areas. Relative brain region sizes were analyzed in separate models using whole brain surface area as a covariate because we were interested in how visual manipulation of the larval environment might influence the proportional size of these regions. In addition to the covariates used for size correction, each model included developmental stage (late larval stage 42–43 and post-metamorphic stage 46) and experimental treatment during the larval period (control and darkened water) as fixed factors as well as the stage*treatment interaction. Tests of model effects used the Wald Chi-square statistic ($\alpha = 0.05$) and the results were explored by assessing estimated marginal means (EMM) of the models. Additionally, least significant difference (LSD) pairwise contrasts were used to explore the significant stage*treatment interaction results. All statistics were computed in SPSS 26 (IBM, Armonk, NY).

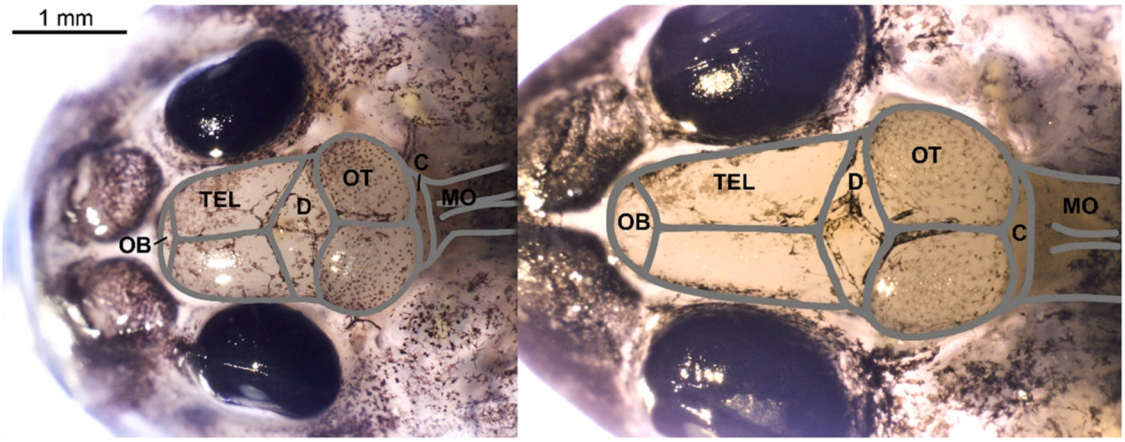


Fig. 1. Dorsal view of fire-bellied toads with exposed brain highlighted by gray outlines. Left: larva at stage 42. Right: post-metamorphic juvenile at stage 46. The exposed dorsal surface area of the brain excluding the medulla oblongata was used to estimate brain size. Brain morphology was assessed by measuring the surface area of both halves of the optic tectum and the telencephalon (including the olfactory bulb). The 1 mm scale bar on the top left applies to both photographs. Abbreviations: C: cerebellum, D: diencephalon, MO: medulla oblongata, OB: olfactory bulb, OT: optic tectum, TEL: telencephalon (excluding olfactory bulb).

3. Results

3.1. Survival

After accounting for larvae removed for morphological measurements, there was a drastic decrease in survival after the first 5 days of development corresponding to larval hatching (Fig. 2). Low survival rates of the offspring of captive-bred amphibians are common (Browne and Zippel, 2007). Fig. 2A shows the survival data and Fig. 2B shows the probabilities of survival used in the log-rank analysis. Larvae reared in darkened water had slightly but significantly higher survival than controls ($\chi^2(1,263) = 4.75$, $p = 0.03$). This finding supports the notion that the dye used to darken water was not toxic at the concentration used. A tendency for development to stall after reaching stage 25 was noted in both treatments. Many individuals passed through this stage very slowly or not at all, a phenomenon that could be related to developmental stasis in larvae that had difficulties feeding (see McKeown et al., 2017). Since stage 25 of development was normally reached at 7 dpf, we considered larvae still showing characteristics of stage 25 at 16 dpf to be in developmental stasis. A total of 8 of the 52 larvae assessed at 16 dpf (15%) were in stasis (3 dark-reared and 5 controls). Some of these larvae remained in stasis up to 25 dpf before they died. These individuals often displayed notable morphological abnormalities. Note that none of the stages impacted by this delay were used for the developmental rate analysis presented next.

3.2. Developmental rate

We found a statistically significant interaction between developmental stage and treatment on the age of larvae (Fig. 3A; Stage: $F_{2,101} = 33.2$, $p < 0.01$; Treatment: $F_{1,101} = 1.3$, $p = 0.26$; Stage*Treatment: $F_{2,101} = 14.9$, $p < 0.01$). Tests of the effect of treatment at each developmental stage revealed an acceleration of development at stage 30–31 ($F_{1,28} = 13.2$, $p < 0.01$) and a delay in development by stage 42–43 ($F_{1,22} = 7.6$, $p = 0.01$) in the darkened water treatment. There was no significant difference in age between darkened water and control treatments at stage 40–41 ($F_{1,53} = 1.3$, $p = 0.26$). This trend suggests a complex effect of the darkened water treatment on developmental rate whereby it accelerates development at first but then slows it down substantially, allowing controls to reach metamorphosis earlier.

When including only specimens at stages 30–31, 40–41 and 42–43 measured alive during selected nightly water changes, there was no difference in snout-vent length between larvae from the darkened water and control treatments when analyzed as a function of developmental

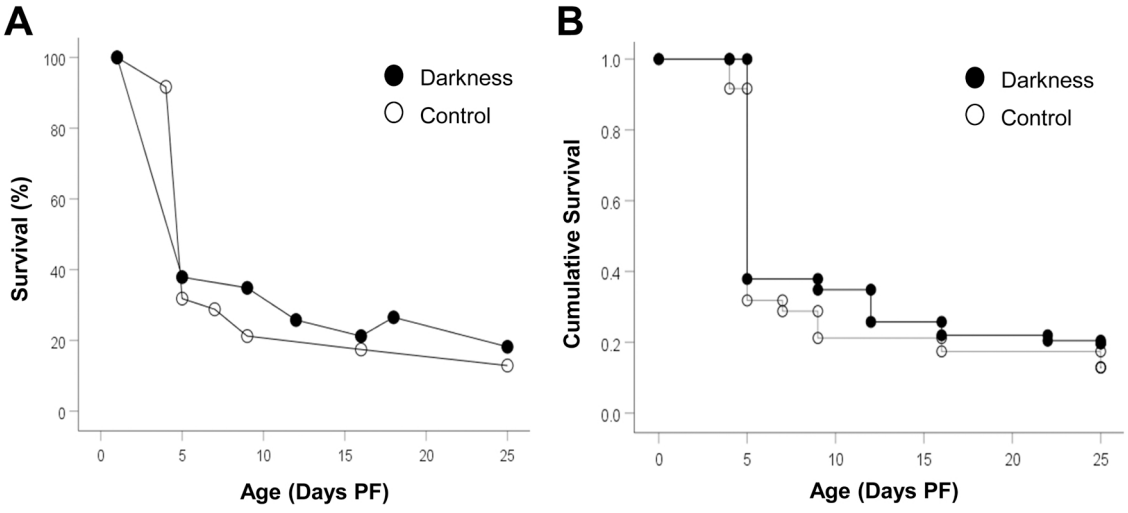


Fig. 2. Survival of fire-bellied toad larvae in darkened water and control treatments. A) Percent survival of larvae by age measured in days post-fertilization (PF). B) Cumulative survival probabilities by age used in the log-rank analysis. Black symbols represent darkened water treatment, while white symbols represent controls.

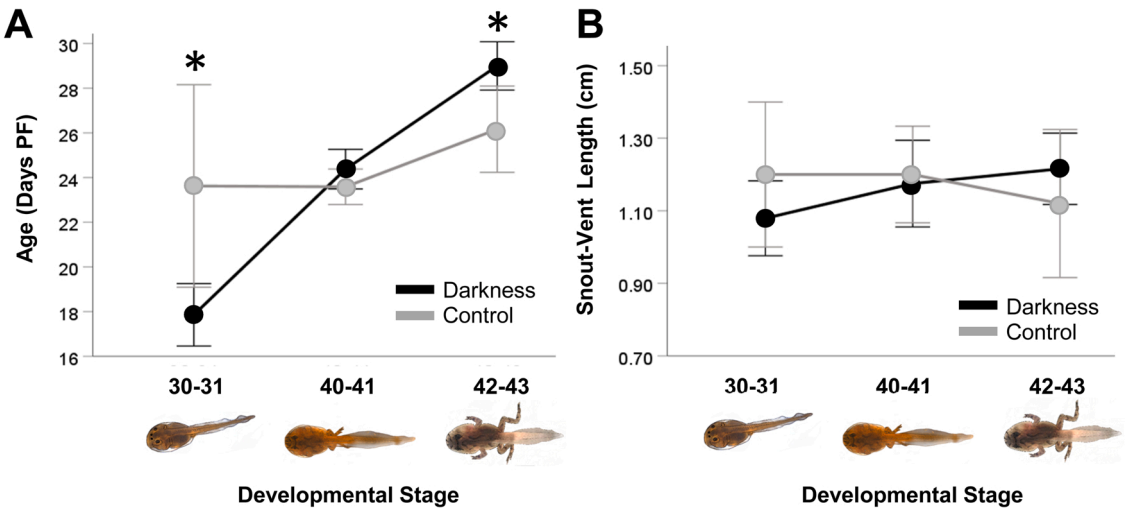


Fig. 3. Effect of darkened water treatment on fire-bellied toad larval development and growth. A) Larval age (days post-fertilization) by developmental stage. B) Larval snout-vent length (cm) by developmental stage. Asterisks indicate statistically significant differences between treatments at a developmental stage. Black symbols and lines represent the darkened water treatment, while gray symbols and lines represent controls. Symbols are means ± 95% confidence intervals. Samples sizes for data presented in panel A) are as follows: Developmental Stage 30–31: Control= 8, Darkness= 21; Developmental Stage 40–41: Control= 17, Darkness= 37; Developmental Stage 42–43: Control= 12, Darkness= 11. Samples sizes for data presented in panel B) are as follows: Developmental Stage 30–31: Control= 7, Darkness= 17; Developmental Stage 40–41: Control= 9, Darkness= 20; Developmental Stage 42–43: Control= 5, Darkness= 9.

stage (Fig. 3B; Stage: $F_{2,61}$ =0.3, p = 0.75; Treatment: $F_{1,61}$ =0.1, p = 0.77; Stage*Treatment: $F_{2,61}$ =1.1, p = 0.35). When fixed specimens at late larval and juvenile stages were included in the analysis of snout-vent length, there was also no effect of treatment at any stage (Treatment: $F_{1,93}$ =2.0, p = 0.16; Stage*Treatment: $F_{3,93}$ =1.2, p = 0.32) and no significant difference in snout-vent length between developmental stages 30–31 to 46 (Stage: $F_{3,93}$ =0.8, p = 0.48).

3.3. Brain size

In addition to the expected positive relationship between body size and brain size (SVL: $\chi^2(1,43) = 14.02$, $P < 0.001$), the interaction between developmental stage and larval visual environment was statistically significant (Fig. 4A; Stage: $\chi^2(1,43) = 6.95$, $P = 0.008$; Treatment: $\chi^2(1,43) = 7.86$, $P = 0.005$; Stage*Treatment: $\chi^2(1,43) = 6.37$, $P = 0.01$). The darkened water treatment produced no difference in relative brain size by the end of the larval period (Treatment LSD contrast: $P = 0.94$) compared with an average 14.4% reduction in brain size due to treatment in post-metamorphic juveniles (Treatment LSD contrast: $P < 0.001$). Relative brain size was greater in post-metamorphic juvenile controls despite no difference in body size between the late larval and post-metamorphic stages sampled (see above), which suggests increased investment in brain growth with metamorphosis. The darkened water treatment appears to have prevented this post-metamorphic surge in brain growth.

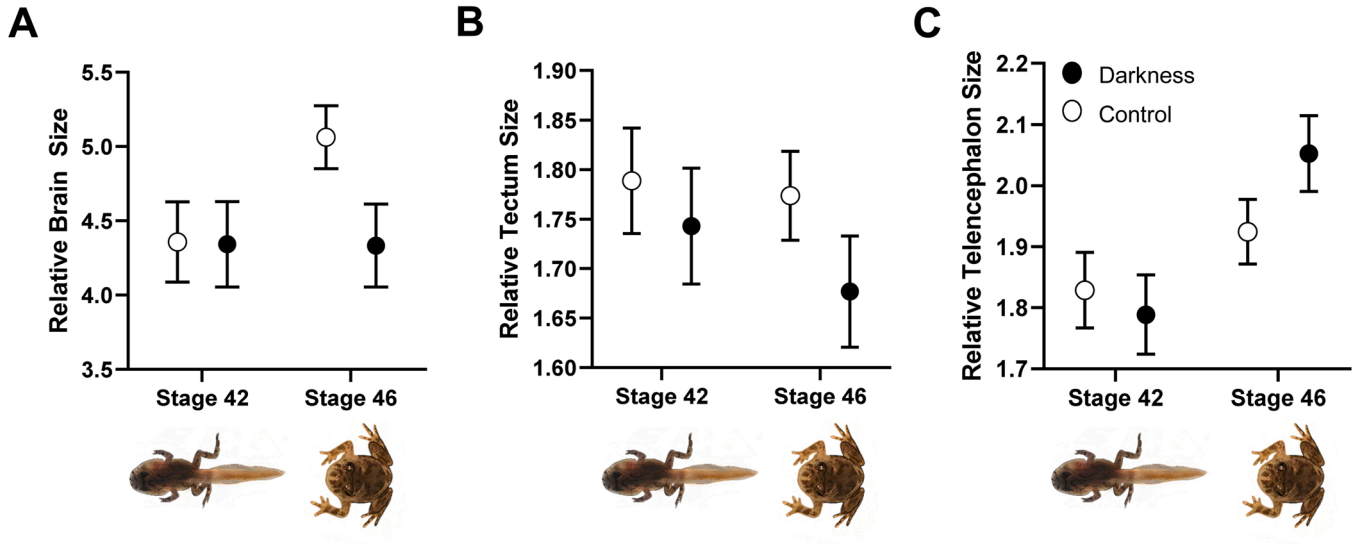


Fig. 4. Effect of metamorphosis and larval darkened water treatment on brain form in late larval (stage 42) and juvenile (stage 46) fire-bellied toads. A) Relative brain size, B) Relative optic tectum size, and C) relative telencephalon size. Black symbols represent darkened water treatment, while white symbols represent controls. Each symbol represents the estimated marginal means of generalized linear models including snout-vent length (A) or whole brain surface area (B-C) as a covariate and associated 95% confidence intervals around the means. Sample sizes are as follows: control – stage 42 (11 in A-B, 10 in C), control – stage 46 (17 in A-B, 15 in C), darkness – stage 42 (10 in all panels), and darkness – stage 46 (10 in all panels).

3.4. Regional brain size

As expected, size of the optic tectum was positively associated with whole brain size (Brain Area: $\chi^2(1,44) = 175.9$, $P < 0.001$). Developmental stage had no effect on relative tectum size (Stage: $\chi^2(1,44) = 2.15$, $P = 0.14$), but the darkened water treatment significantly reduced relative size of this brain region (Treatment: $\chi^2(1,44) = 6.42$, $P = 0.01$; Fig. 4B). Although the interaction between developmental stage and larval visual environment was not statistically significant (Stage*Treatment: $\chi^2(1,44) = 0.93$, $P = 0.34$), a trend for greater tectum size reduction due to the darkened water treatment was noted in juveniles (average 5.5% reduction) compared to the late larval stage (2.6% reduction).

As with the optic tectum, size of the telencephalon was positively associated with whole brain size (Brain Area: $\chi^2(1,41) = 319.9$, $P < 0.001$). Unlike the tectum, developmental stage had an important effect on relative telencephalon size (Stage: $\chi^2(1,41) = 32.47$, $P < 0.001$; Fig. 4C), increasing contribution of the telencephalon to brain size by 9.9% in juveniles compared to the late larval stage. This suggests that metamorphosis in the fire-bellied toad is associated with enhanced growth of the telencephalon. A statistically significant interaction between developmental stage and larval visual environment was also seen (Stage*Treatment: $\chi^2(1,41) = 7.79$, $P = 0.005$) because the average 6.7% increase in relative telencephalon size due to the darkened water treatment was limited to juveniles (Treatment LSD contrasts: late larval, $P = 0.37$; juveniles, $P = 0.003$).

4. Discussion

4.1. Developmental rate

Our results revealed an effect of larval visual restriction on developmental rate. Larvae reared in darkened water developed faster than controls initially but delayed their development closer to metamorphosis so much that controls achieved metamorphosis significantly faster than larvae in the darkened water treatment. Plasticity in developmental rate is a well-established phenomenon in anuran larvae (Richter-Boix et al., 2011). Typically, changes in the timing of key developmental events have been studied in the context of desiccation and predation risk, with plasticity constrained by trade-offs between development and growth rates (Richter-Boix et al., 2011). This trade-off normally results in size variation at metamorphosis, which has been shown to affect size at reproduction, and therefore, fitness (Travis, 1984; Werner, 1986; Bekhet et al., 2014). Hence, developmental rate is inherently linked with post-metamorphic performance. A much smaller number of studies have looked at the effects of light and visual environment on developmental rate plasticity. Consistently, these studies showed that light availability promotes faster growth and development (Eichler and Gray, 1976; Borah et al., 2018; Ruchin, 2021). On the other hand, continuous darkness has been shown to delay development and growth via increased melatonin levels, which inhibit the thyroid hormone axis, a pivotal developmental regulatory hormone in amphibians (Delgado et al., 1987; Wright et al., 2000). Therefore, the developmental delay observed in fire-bellied toad larvae reared in darkened water could have been expected. Still, the developmental delay was preceded by an acceleration of developmental rate in earlier stages of development before slowing down. This finding, while unexpected, suggests that the relationship between the light environment and development is more complex than previously predicted and that some developmental periods during larval development in *B. orientalis* are more sensitive to light. The putative relationship of darkness sensitivity to thyroid hormone signaling should be investigated in future studies as it could shed light on the mechanisms underlying this process. However, it should be noted that the quick transition of control larvae from stages 30–42 limited our ability to assess them in the earlier stages of development on measurement nights, resulting in a lower sample size for controls at stage 30–31. This is an important limitation to the conclusion of a complex effect of larval visual restriction on developmental rate that will require confirmation in the future.

The developmental delay we observed in later stages was not accompanied by an increase in larval size. It is possible that larvae simply did not access sufficient nutrients to capitalize on delayed development with increased metamorphic size despite a regular feeding schedule. Alternatively, energy allocation decisions may have been made towards traits that were not measured in this study, such as energy retention for gamete production. Such carry-over effects due to selective energy allocation have been extensively discussed in the literature and are ultimately linked to the endocrine control of development in anurans (Travis, 1984; Werner, 1986; Beachy, 2001; Bender et al., 2018).

4.2. Distinct mechanisms of change in brain size

When assessing the effect of larval visual restriction on brain form, we found that larvae reared in darkened water showed no increase in relative brain size or proportional tectum size with metamorphosis, but an increase in proportional size of the telencephalon surpassing the one seen in controls followed their metamorphosis. The latter suggests substantial reallocation of brain tissue growth to the telencephalon after metamorphosis in juveniles that had been visually restricted during the larval period. The global latent effect of larval visual restriction on brain size and regional latent effect on telencephalon growth stand in contrast to the plastic effect on optic tectum size seen in larvae, which persisted after metamorphosis. Differences in the timing of effects of the larval visual environment on brain growth across treatments and brain regions suggest that brain size plasticity can be achieved through different mechanisms in anurans.

Anuran metamorphosis is characterized by a reorganization of the nervous system involving changes in gross morphology and connectivity of the brain (Denver, 1998; Horowitz and Simmons, 2007; 2010). The latent effect of larval environment favoring telencephalon growth at the expense of other parts of the brain that we observed suggests regional priming of the ability to respond to cues of metamorphosis. Hormonal changes accompanying metamorphosis are good candidate mechanisms to mediate change in telencephalon size. Pioneer work leading to the discovery of thyroid-stimulating hormone led to the observation that the brain of thyroidectomized anurans retains its larval form and that supplementation of thyroid hormones can induce metamorphic-like changes in brain morphology before the onset of metamorphosis (Allen, 1924). These effects of thyroid hormone on the relative size of brain parts involve modulation of cell proliferation and differentiation, neurite growth, and apoptosis (Denver, 1998; Coen et al., 2007; Thompson and Cline, 2016; Wen et al., 2019). Interestingly, such effects on brain growth are not limited to thyroid hormones. Circulating prolactin and corticosteroid hormone levels surge at metamorphosis in anurans (Clemons and Nicoll, 1977; Jolivet Jaudet and Leloup Hatey, 1984) while growth hormone levels temporarily drop at that same time (Buckbinder and Brown, 1993). Exposure of frog larvae to corticosterone can also influence brain morphology by increasing size of the diencephalon (Cha et al., 2021). Additionally, frog larvae injected with prolactin or growth hormone show altered levels of brain cell proliferation, with distinct acute or latent effects depending on hormonal treatment (Hunt and Jacobson, 1970). These two hormones slow or accelerate development of some larval structures without influencing the timing of metamorphosis in anurans (Delidow, 1989; Huang and Brown, 2000a; 2000b). Therefore, the complexity of hormonal fluctuations at metamorphosis could mediate the distinct types of latent effects (i.e. global and regional) of the larval visual environment manipulation on brain growth that we observed.

Plasticity of optic tectum size showed a different pattern because this brain region was already smaller by the end of the larval period after treatment with darkened water and the smaller tectum size remained after metamorphosis in juveniles exposed to normal illumination. The

optic tectum could be particularly susceptible to larval environmental conditions, as a larval plasticity of tectum growth was also noted with manipulation of rearing density (Gonda et al., 2010; Trokovic et al., 2011). Could larval tectum growth be activity-dependent? Previous studies of developing retinotectal pathways in anurans showed that visual stimulation is not needed for normal development of these connections (Jacobson, 1971; Keating et al., 1986). Instead, visual experience-dependent plasticity of tectal circuits has been implicated in the refinement of visual behavioral responses during anuran development (e.g. Dong et al., 2009). Interestingly, visual stimulation increases neuronal differentiation in the tectum of *Xenopus laevis* and *Danio rerio* larvae while visual deprivation appears to maintain tectal cells in the progenitor pool (Sharma and Cline, 2010; Hall and Tropepe, 2018). This suggests that growth of tectum size could be due to visually-induced greater differentiation of neurons, but the cellular mechanisms of tectum size plasticity remain to be investigated in anurans. Of further interest, Denver et al. (2009) showed that thyroid hormone-sensitive cell proliferation is highest in the developing anuran tectum until mid-prometamorphosis, after which it drops off precipitously. Thereafter, proliferation continues in other brain areas, but it is not sensitive to thyroid hormone treatment. Again, this suggests high sensitivity of tectum growth during the larval period corresponding to the environmentally induced plastic effects seen with visual restriction (current study) and density manipulation (Gonda et al., 2010; Trokovic et al., 2011). The lack of tectum growth response after metamorphosis is in accord with a concurrent drop in brain cell proliferation (Denver et al., 2009) and the carry-over effect of treatment observed here.

4.3. Sensory environments and brain morphology

What could be the functional consequences of developmental brain morphology plasticity in anurans? The optic tectum is involved in orienting and avoidance responses triggered by various stimuli of different sensory modalities, with a major role of vision (Dean et al., 1989; Northmore, 2011). The telencephalon includes the main and accessory olfactory bulbs in its rostral part, which respectively receive primary olfactory and vomeronasal input from peripheral receptor neurons (Manzini and Schild, 2010). The rest of the telencephalon is heavily targeted by efferents from the bulbs and thus is also important for integration of chemosensory information (Laberge and Roth, 2007; Roth and Laberge, 2011). In a very general sense, Jerison's (1973) principle of proper mass posits that the size of a neural structure reflects the complexity of behaviors that it mediates. Therefore, one could predict that dark-reared larvae with an enlarged telencephalon would be better suited to rely on chemosensory information to guide behavior, which also would be appropriate for life conditions unfavorable to vision. This prediction would be better supported by a specific enlargement of the olfactory bulbs, which could only be assessed using a more accurate stereological approach (e.g. histology) in these developing anuran brains. The reduction in tectum size in dark-reared larvae would contribute energy savings by reduced reliance on the energetically costly visual system (Niven and Laughlin, 2008). Visual manipulation of larval rearing environment with a non-toxic water dye offers an easy method to produce animals with variable brain morphology phenotypes in anurans (present study) and fish (Pike et al., 2018), which could be used to assess the generality of Jerison's principle using a variety of behavioral and cognitive tests. Additionally, it offers the opportunity to study the variable mechanisms of change in brain size that happen during larval exposure and thereafter as latent effects in juveniles. Although exposure to complete darkness during the larval period is unlikely to happen to anurans under natural conditions, our findings suggest that ecologically relevant environmental conditions during development, such as variation in illumination due to vegetation cover, could induce more subtle changes in brain form with potential impacts on fitness when early and late life stages share predictable environmental conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Discovery Grants of the Natural Sciences and Engineering Research Council of Canada to F. Laberge and A. Heyland.

References

Abraham, R.K., Mathew, J.K., Raju, D.V., Rao, R., Zachariah, A., 2018. Reproduction and metamorphosis in the *Myristica* Swamp tree frog, *Mercurana myristicapalustris* (Anura: *Rhacophoridae*). PeerJ 6, 1–19.

Allen, B.M., 1924. Brain development in anuran larvae after thyroid or pituitary gland removal. Endocrinology 8, 639–651.

Beachy, C.K., 2001. Effects of growth history and exogenous thyroxine on size and age at metamorphosis in the toad *Bufo americanus*. Copeia 2001, 829–834.

Bekhet, G.A., Abdou, H.A., Dekinesh, S.A., Hussein, H.A., Sebiae, S.S., 2014. Biological factors controlling developmental duration, growth and metamorphosis of the larval green toad, *Bufo viridis viridis*. J. Basic Appl. Zool. 67, 67–82.

Bender, M.C., Hu, C., Pelletier, C., Denver, R.J., 2018. To eat or not to eat: ontogeny of hypothalamic feeding controls and a role for leptin in modulating life-history transition in amphibian tadpoles. Proc. Royal Soc. B 285, 20172784.

Bishop, C.D., Ereyilmaz, D.F., Flatt, T., Georgiou, C.D., Hadfield, M.G., Heyland, A., Hodin, J., Jacobs, M.W., Maslakova, S.A., Pires, A., Reitzel, A.M., Santagata, S., Tanaka, K., Youson, J.H., 2006. What is metamorphosis? Integr. Comp. Biol. 46, 655–661.

Borah, B.K., Hauzel, L., Renthlei, Z., Trivedi, A.K., 2018. Photic and non-photic regulation of growth, development, and metamorphosis in giant tree frog (*Rhacophorus maximus*) tadpoles. Biol. Rhythm Res. 49, 955–967.

Bredeweg, E.M., Urbina, J., Morzillo, A.T., Garcia, T.S., 2019. Starting on the right foot: carryover effects of larval hydroperiod and terrain moisture on post-metamorphic frog movement behavior. Front. Ecol. Evol. 7, 97. https://doi.org/10.3389/fevo.2019.00097.

Browne, R.K., Zippel, K., 2007. Reproduction and larval rearing of amphibians. ILAR J. 48, 214–234.

Buckbinder, L., Brown, D.D., 1993. Expression of the *Xenopus laevis* prolactin and thyrotropin genes during metamorphosis. Proc. Natl. Acad. Sci. USA 90, 3820–3824.

Campero, M., De Block, M., Ollevier, F., Stoks, R., 2008. Metamorphosis offsets the link between larval stress, adult asymmetry and individual quality. Funct. Ecol. 22, 271–277.

Cha, E.S., Uhrin, M.T., McLelland, S.J., Woodley, S.K., 2021. Brain plasticity in response to short-term exposure to corticosterone in larval amphibians. Can. J. Zool 99. https://doi.org/10.1139/cjz-2021-0031.

Clemons, G.K., Nicoll, C.S., 1977. Development and preliminary application of a homologous radioimmunoassay for bullfrog prolactin. Gen. Comp. Endocrinol. 32, 531–535.

Coen, L., Le Blay, K., Rowe, I., Demeneix, B.A., 2007. Caspase-9 regulates apoptosis/proliferation balance during metamorphic brain remodeling in *Xenopus*. Proc. Natl. Acad. Sci. USA 104, 8502–8507.

Coolen, I., Ward, A.J.W., Hart, P.J.B., Laland, K.N., 2005. Foraging nine-spined sticklebacks prefer to rely on public information over simpler social cues. Behav. Ecol. 16, 865–870.

Crean, A.J., Monro, K., Marshall, D.J., 2011. Fitness consequences of larval traits persist across the metamorphic boundary. Evolution 65, 3079–3089.

Dantzer, B., Newman, A.E.M., Boonstra, R., Palme, R., Boutin, S., Humphries, M.M., McAdam, A.G., 2013. Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. Science 340, 1215–1217.

Dean, P., Redgrave, P., Westby, G.W.M., 1989. Event or emergency? Two response systems in the mammalian superior colliculus. Trends Neurosci. 12, 137–147.

Delgado, M.J., Gutiérrez, P., Alonso-Bedate, M., 1987. Melatonin and photoperiod alter growth and larval development in *Xenopus laevis* tadpoles. Comp. Biochem. Physiol. A 86, 417–421.

Delidow, B.C., 1989. Reevaluation of the effects of growth hormone and prolactin on anuran tadpole growth and development. J. Exp. Zool. 249, 279–283.

Denver, R.J., 1998. The molecular basis of thyroid hormone-dependent central nervous system remodeling during amphibian metamorphosis. Comp. Biochem. Physiol. C 119, 219–228.

Denver, R.J., Hu, F., Scanlan, T.S., Furlow, J.D., 2009. Thyroid hormone receptor subtype specificity for hormone-dependent neurogenesis in *Xenopus laevis*. Dev. Biol. 326, 155–168.

Dong, W., Lee, R.H., Xu, H., Yang, S., Pratt, K.G., Cao, V., Song, Y.-K., Nurmikko, A., Aizenman, C.D., 2009. Visual avoidance in *Xenopus* tadpoles is correlated with the maturation of visual responses in the optic tectum. J. Neurophysiol. 101, 803–815.

Eichler, V.B., Gray Jr, L.S., 1976. The influence of environmental lighting on the growth and prometamorphic development of larval *Rana pipiens*. Dev. Growth Differ. 18, 177–182.

Fadl, A.E.A., Mahfouz, M.E., El-Gamal, M.M.T., Heyland, A., 2019. Onset of feeding in juvenile sea urchins and its relation to nutrient signaling. Invertebr. Reprod. Dev. 63, 11–22.
Frank, D.F., Brander, S.M., Hasenbein, S., Harvey, D.J., Lein, D.J., Geist, J., Connon, R.E., 2019. Developmental exposure to environmentally relevant concentrations of bifenthrin alters transcription of mTOR and ryanodine receptor-dependent signaling molecules and impairs predator avoidance behavior across early life stages in inland silversides (*Menidia beryllina*). Aquat. Toxicol. 206, 1–13.
Garcia, T.S., Urbina, J.C., Bredeweg, E.M., Ferrari, M.C.O., 2017. Embryonic learning and developmental carry-over effects in an invasive anuran. Oecologia 184, 623–631.
Girish, S., Saidapur, S.K., 2003. Density-dependent growth and metamorphosis in the larval bronze frog *Rana temporalis* is influenced by genetic relatedness of the cohort. J. Biosci 28, 489–496.
Gonda, A., Trokovic, N., Herczeg, G., Laurila, A., Merilä, J., 2010. Predation- and competition-mediated brain plasticity in *Rana temporaria* tadpoles. J. Evol. Biol. 23, 2300–2308.
Gosner, K.L., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16, 183–190.
Hall, Z.J., Tropepe, V., 2018. Visual experience facilitates BDNF-dependent adaptive recruitment of new neurons in the postembryonic optic tectum. J. Neurosci. 38, 2000–2014.
Hall, B.K., Wake, M.H., 1999. The Origin And Evolution of Larval Forms. Academic Press, San Diego.
Hart, M.W., Strathmann, R.R., 1994. Functional consequences of phenotypic plasticity in echinoid larvae. Biol. Bull. 186, 291–299.
Hayes, T.B., Anderson, L.L., Beasley, V.R., de Solla, S.R., Iguchi, T., Ingraham, H., Kestemont, P., Kniewald, J., Kniewald, Z., Langlois, V.S., Luque, E.H., McCoy, K.A., Muñoz-de-Toro, M., Oka, T., Oliveira, C.A., Orton, F., Ruby, S., Suzawa, M., Tavera-Mendoza, L.E., Trudeau, V.L., Victor-Costa, A.B., Willingham, E., 2011. Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. J. Steroid Biochem. Mol. Biol. 127, 64–73.
Heyland, A., Moroz, L.L., 2006. Signaling mechanisms underlying metamorphic transitions in animals. Integr. Comp. Biol. 46, 743–759.
Horowitz, S.S., Simmons, A.M., 2007. Dynamic visualization of the developing nervous system of the bullfrog *Rana catesbeiana*. Brain Res. 1157, 23–31.
Horowitz, S.S., Simmons, A.M., 2010. Development of tectal connectivity across metamorphosis in the bullfrog *(Rana catesbeiana)*. Brain Behav. Evol. 76, 226–247.
Huang, H., Brown, D.D., 2000a. Prolactin is not a juvenile hormone in *Xenopus laevis* metamorphosis. Proc. Natl. Acad. Sci. USA 97, 195–199.
Huang, H., Brown, D.D., 2000b. Overexpression of *Xenopus laevis* growth hormone stimulates growth of tadpoles and frogs. Proc. Natl. Acad. Sci. USA 97, 190–194.
Hunt, R.K., Jacobson, M., 1970. Brain enhancement in tadpoles: increased DNA concentration after somatotrophin or prolactin. Science 170, 342–344.
Jacobson, M., 1971. Absence of adaptive modification in developing retinotectal connections in frogs after visual deprivation or disparate stimulation of the eyes. Proc. Natl. Acad. Sci. USA 68, 528–532.
Jerison, H.J., 1973. The Evolution of The Brain and Intelligence. Academic Press, New York.
Jolivet Jaudet, G., Leloup Hatey, J., 1984. Variations in aldosterone and corticosterone plasma levels during metamorphosis in *Xenopus laevis* tadpoles. Gen. Comp. Endocrinol. 56, 59–65.
Keating, M.J., Grant, S., Dawes, E.A., Nanchahal, K., 1986. Visual deprivation and the maturation of the retinotectal projection in *Xenopus laevis*. J. Embryol. Exp. Morphol. 91, 101–115.
Laberge, F., Roth, G., 2007. Organization of the sensory input to the telencephalon in the fire-bellied toad, *Bombina orientalis*. J. Comp. Neurol. 502, 55–74.
Lewis, C., Ford, A.T., 2012. Infertility in male aquatic invertebrates: a review. Aquat. Toxicol. 120–121, 79–89.
Liao, W.B., Lou, S.L., Zeng, Y., Merilä, J., 2015. Evolution of anuran brains: disentangling ecological and phylogenetic sources of variation. J. Evol. Biol. 28, 1986–1996.
Manzano, A.S., Herrel, A., Fabre, A.C., Abdala, V., 2017. Variation in brain anatomy in frogs and its possible bearing on their locomotor ecology. J. Anat. 231, 38–58.
Manzini, I., Schild, D., 2010. Olfactory coding in larvae of the African clawed frog *Xenopus laevis*. In: Menini, A. (Ed.), The Neurobiology of Olfaction. CRC Press, Taylor Francis, Boca Raton.
Marshall, D.J., Morgan, S.G., 2011. Ecological and evolutionary consequences of linked life-history stages in the sea. Curr. Biol. 21, R718–R725.
McGraw, K.O., Wong, S.P., 1996. Forming inferences about some intraclass correlation coefficients. Psychol. Methods 1, 30–46.
McKeown, C.R., Thompson, C.K., Cline, H.T., 2017. Reversible developmental stasis in response to nutrient availability in the *Xenopus laevis* central nervous system. J. Exp. Biol. 220, 358–368.
Miner, B.G., Vonesh, J.R., 2004. Effects of fine grain environmental variability on morphological plasticity. Ecol. Lett 7, 794–801.
Moran, N.A., 1994. Adaptation and constraint in the complex life cycles of animals. Annu. Rev. Ecol. Evol. Syst. 25, 573–600.
Nieuwkoop, P.D., Faber, J., 1956. Normal table of *Xenopus laevis* (Daudin). A systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. North-Holland Publ,, Co., Amsterdam.
Niven, J.E., Laughlin, S.B., 2008. Energy limitation as a selective pressure on the evolution of sensory systems. J. Exp. Biol 211, 1792–1804.
Northmore, D., 2011. Optic tectum. In: Farrell, A.P., Stevens, E.D., Cech, J.J., Richards, J. G. (Eds.), Encyclopedia of Fish Physiology. Academic Press, Boston, pp. 131–142.
Padmanabhan, V., Cardoso, R.C., Puttabyatappa, M., 2016. Developmental programming, a pathway to disease. Endocrinology 157, 1328–1340.
Park, C.J., Ahn, H.M., Cho, S.C., Kim, T., Oh, J., Ahn, H.K., Chun, S., Gye, M.C., 2014. Developmental toxicity of treated municipal wastewater effluent on *Bombina orientalis* (Amphibia: Anura) embryos. Environ. Toxicol. Chem. 33, 954–961.
Pechenik, J.A., Wendt, D.E., Jarrett, J.N., 1998. Metamorphosis is not a new beginning: larval experience influences juvenile performance. BioScience 48, 901–910.
Pechenik, J.A., 2006. Larval experience and latent effects—metamorphosis is not a new beginning. Integr. Comp. Biol. 46, 323–333.
Pike, T.W., Ramsey, M., Wilkinson, A., 2018. Environmentally induced changes to brain morphology predict cognitive performance. Philos. Trans. R. Soc. Lond. B Biol. Sci. 373, 1–8.
Porseryd, T., Larsson, J., Kellner, M., Bollner, T., Dinnétz, P., Hällström, I.P., 2019. Altered non-reproductive behavior and feminization caused by developmental exposure to 17α-ethinylestradiol persist to adulthood in three-spined stickleback (*Gasterosteus aculeatus*). Aquat. Toxicol. 207, 142–152.
Relya, R.A., 2007. Getting out alive: how predators affect the decision to metamorphose. Oecologia 152, 389–400.
Richter-Boix, A., Tejedo, M., Rezende, E.L., 2011. Evolution and plasticity of anuran larval development in response to desiccation. A comparative analysis. Ecol. Evol 1, 15–25.
Rohr, J.R., Palmer, B.D., 2005. Aquatic herbicide exposure increases salamander desiccation risk eight months later in a terrestrial environment. Environ. Toxicol. Chem. 24, 1253–1258.
Roth, F.C., Laberge, F., 2011. High convergence of olfactory and vomeronasal influence in the telencephalon of the terrestrial salamander *Plethodon shermani*. Neuroscience 177, 148–158.
Ruchin, A.B., 2021. The effect of illumination and light spectrum on growth and larvae development of *Pelophylax ridibundus* (Amphibia: Anura). Biol. Rhythm Res. 52, 307–318.
Salthe, S.N., 1963. The egg capsules in the amphibia. J. Morphol 113, 161–171.
Sharma, P., Cline, H.T., 2010. Visual activity regulates neural progenitor cells in developing *Xenopus* CNS through Musashi1. Neuron 68, 442–455.
Sheriff, M.J., Love, O.P., 2013. Determining the adaptive potential of maternal stress. Ecol. Lett. 16, 271–280.
Shumway, W., 1940. Stages in the normal development of *Rana pipiens*. Anat. Rec. 78, 139–147.
Sive, H.L., Grainger, R.M., Harland, R.M., 2007. Dejellying *Xenopus laevis* embryos. Cold Spring Harb. Protoc. https://doi.org/10.1101/pdb.prot4731.
Strathmann, R.R., Fenaux, L., Sewell, A.T., Strathmann, M.F., 1993. Abundance of food affects relative size of larval and postlarval structures of a molluscan veliger. Biol. Bull. 185, 232–239.
Sussman, P., Betz, T.W., 1978. Embryonic stages: morphology, timing, and variance in the toad *Bombina orientalis*. Can. J. Zool 56, 1540–1545.
Taylor, G.M., Nol, E., Boire, D., 1995. Brain regions and encephalization in anurans: adaptation or stability? Brain Behav. Evol. 45, 96–109.
Thompson, C.K., Cline, H.T., 2016. Thyroid hormone acts locally to increase neurogenesis, neuronal differentiation, and dendritic arbor elaboration in the tadpole visual system. J. Neurosci. 36, 10356–10375.
Travis, J., 1984. Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. Ecology 65, 1155–1160.
Trokovic, N., Gonda, A., Herczeg, G., Laurila, A., Merilä, J., 2011. Brain plasticity over the metamorphic boundary: carry-over effect of larval environment on froglet brain development. J. Evol. Biol 24, 1380–1385.
Wen, L., He, C., Sifuentes, C.J., Denver, R.J., 2019. Thyroid hormone receptor alpha is required for thyroid hormone-dependent neural cell proliferation during tadpole metamorphosis. Front. Endocrinol. 10, 396. https://doi.org/10.3389/fendo.2019.00396.
Werner, E.E., 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. Am. Nat. 128, 319–341.
Woodley, S.K., Mattes, B.M., Yates, E.K., Relya, R.A., 2015. Exposure to sublethal concentrations of a pesticide or predator cues induces changes in brain architecture in larval amphibians. Oecologia 179, 655–665.
Wright, M.L., Cuthbert, K.L., Donohue, M.J., Solano, S.D., Proctor, K.L., 2000. Direct influence of melatonin on the thyroid and comparison with prolactin. J. Exp. Zool. 286, 625–631.