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Predation- and competition-mediated brain plasticity in *Rana temporaria* tadpoles

A. GONDA*, N. TROKOVIC*, G. HERCZEG*, A. LAURILA† & J. MERILÄ*

*Ecological Genetics Research Unit, Department of Biosciences, University of Helsinki, Helsinki, Finland
†Population and Conservation Biology/Department of Ecology and Evolution, Evolutionary Biology Center, Uppsala University, Uppsala, Sweden

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Abstract

An increasing number of studies have demonstrated phenotypic plasticity in brain size and architecture in response to environmental variation. However, our knowledge on how brain architecture is affected by commonplace ecological interactions is rudimentary. For example, while intraspecific competition and risk of predation are known to induce adaptive plastic modifications in morphology and behaviour in a wide variety of organisms, their effects on brain development have not been studied. We studied experimentally the influence of density and predation risk on brain development in common frog (*Rana temporaria*) tadpoles. Tadpoles grown at low density and under predation risk developed smaller brains than tadpoles at the other treatment combinations. Further, at high densities, tadpoles developed larger optic tecta and smaller medulla oblongata than those grown at low densities. These results demonstrate that ecological interactions – like intraspecific competition and predation risk – can have strong effects on brain development in lower vertebrates.

Introduction

Phenotypic plasticity is an important and taxonomically widespread phenomenon providing means to cope with environmental heterogeneity in an adaptive fashion (e.g. Schlichting & Pigliucci, 1998; Miner *et al.*, 2005; Callahan *et al.*, 2008). The majority of plasticity studies have focused on behavioural, morphological and life history traits; however, the plasticity of internal organs has only recently begun to receive increasing attention (e.g. Piersma & Drent, 2003).

The brain is the centre of the nervous system and, as such, an extremely important organ in vertebrates. Laboratory studies have demonstrated brain plasticity at different neuroanatomical levels and life stages in several taxa, including mammals, fishes and reptiles (e.g. Kempermann *et al.*, 1997; Font *et al.*, 2001; Zupanc, 2001). For instance, there is strong evidence for seasonal plasticity in the size of certain neural structures (Nottebohm, 1981; Tramontin & Brenowitz, 2000) and that mental and physical training (e.g. Gould *et al.*, 1999; Rhode *et al.*, 2003) can influence neural architecture. The effects of environmental complexity (reviewed in Van Praag *et al.*, 2000) and social environment (Fowler *et al.*, 2002; Sorensen *et al.*, 2007; Adar *et al.*, 2008; Gonda *et al.*, 2009) on brain development have also been demonstrated. Two lines of evidence suggest that these plastic modifications can be of adaptive value. First, previous studies in brain development have demonstrated that those parts of the brain that are likely to be important in a particular context develop more than those of less importance (Kihslinger & Nevitt, 2006; Kihslinger *et al.*, 2006; Lisney *et al.*, 2007). Second, as the brain is the most expensive tissue to develop and maintain (Aiello & Wheeler, 1995) energetic constraints should impose strong selection against nonadaptive modifications of brain. However, while environmentally induced plasticity in brain development appears to be common, studies of the ecological and evolutionary relevance of this plasticity are slow to accumulate. The understanding of how ecological interactions may modify brain architecture is almost nonexistent, for example, we are not aware of any studies investigating the effect of

Correspondence: Nina Trokovic, Ecological Genetics Research Unit, Department of Biosciences, University of Helsinki, PO Box 65, Helsinki FI-00014, Finland.
Tel.: +358 9 191 57802; fax: +358 9 191 57694;
e-mail: trokovic@mappi.helsinki.fi

predation risk or competition on brain architecture. Further, factorial experiments investigating independent and joint effects of different treatments are lacking. Studies incorporating these major ecological factors would be valuable in understanding the importance of plasticity in brain architecture for natural populations.

Predation risk and intraspecific competition often induce plastic modifications in morphology and behaviour in a variety of organisms (Miner *et al.*, 2005; Callahan *et al.*, 2008). Amphibian larvae show strong plastic responses to intraspecific competition and exhibit a wide range of phenotypic plasticity in response to predation risk (Miner *et al.*, 2005). While the tadpoles' responses can vary among specific predator types (e.g. Van Buskirk, 2001; Laurila *et al.*, 2006), predation risk posed by larval dragonflies tends to induce smaller body size, deeper tail and tail muscle, and lowered activity (e.g. Skelly & Werner, 1990; McCollum & Van Buskirk, 1996; Van Buskirk & Relyea, 1998). These modifications are adaptive, as they increase survival under predation risk (McCollum & Van Buskirk, 1996; Van Buskirk & Relyea, 1998). Intense intraspecific competition, on the contrary, induces larger bodies, shallower tails and higher activity (Relyea, 2002). Modifications of other structures and internal organs, including mouthparts and the gut, have also been reported (Relyea & Auld, 2004, 2005). While these competitor-induced modifications increase the competitive ability of tadpoles, they also increase vulnerability to predation (Relyea, 2002). Although the brain is important for processing sensory stimuli key to the detection and behavioural avoidance of predators, as well as in feeding (e.g. Köhler & Moyà-Solà, 2004), whether predation risk or competitors induce plastic changes in the brain architecture remains unexplored in tadpoles or in other taxa.

The aim of this study was to explore the potential effects of predation risk and intraspecific competition on brain development of common frog (*Rana temporaria*) tadpoles. Previous studies have shown that *R. temporaria* tadpoles express behavioural and morphological plasticity in response to both predators and competitors (e.g. Laurila *et al.*, 1998, 2004; Van Buskirk, 2001; Teplitsky & Laurila, 2007). We raised tadpoles in a two-factor experiment, manipulating levels of intraspecific competition and predation risk, and asked the following questions: Does intraspecific competition and/or predation risk influence brain size of tadpoles? Which brain regions are affected by these treatments? Do predation risk and competition have synergistic effects on brain size? Are brain phenotypes correlated with the treatment-induced morphological phenotypes? We considered two possible levels of effects on brain development. First, considering energetic constraints (the brain is the most expensive tissue to develop and maintain, e.g. Aiello & Wheeler, 1995), we predicted that (i) high intraspecific density that was likely to be resulted in high competition for low per capita food resources would imply an energetic constraint on overall brain development and (ii) the presence of a predator would also constrain brain development, especially at low density where foraging activity is reduced because of the perceived high per capita predation risk. Second, considering that those parts of the brain that are likely to be important in a particular context develop more than those of less importance (e.g. Lisney *et al.*, 2007), we predicted that the size of brain structures related to perception or learning (e.g. telencephalon, optic tectum) will increase with increased social complexity and higher competition, as well as in the presence of predators.

Materials and methods

Experimental animals

We collected *R. temporaria* eggs from a population in central Sweden (Stora Almby, Uppsala municipality, Sweden, 59°51′N, 17°28′E, and altitude 40 m) on 9 April 2008. Approximately 500 freshly laid eggs were collected from each of 12 families and immediately transported to the laboratory in Uppsala. The eggs were reared in family-specific 3 L vials containing reconstituted soft water (APHA1985; changed every 3 days) at a constant temperature of 18 °C. Hatched tadpoles were maintained in these vials until they reached developmental stage 25 (complete absorption of external gills; Gosner, 1960). Late-instar dragonfly larvae (*Aeshna* sp.), which are voracious predators of the tadpoles, were collected from ponds near Uppsala and used as predators in the experiment.

Experimental design

The experiments were conducted in plastic tanks ($36 \times 40 \times 90$ cm) placed in a fenced field in Uppsala. The tanks were established 2 weeks before the beginning of the experiments, to allow algal growth. The tanks were filled with 90 L of water, 10 g of dried leaves (*Betula* sp., *Populus tremula*) and 4 g of rabbit pellets, inoculated with 1 L of pond water, and covered with mosquito net to prevent colonization by insects. On April 21, we pooled 100 seemingly healthy tadpoles from each of the 12 clutches into a bucket and then allocated the appropriate number of tadpoles to each tank.

We manipulated total density of tadpoles (high density = 50 tadpoles/tank; low density = 10 tadpoles/tank; these densities are within the natural range of tadpole density in *R. temporaria* (Laurila, 1998; A. Laurila, personal observation) and predator presence. In the predator treatment, one dragonfly larva was placed in a cylindrical cage (diameter 8 cm; height 21 cm) made of transparent plastic film with a double net bottom (mesh size 1.5 mm) and hung 6 cm over the tank bottom. This allowed the tadpoles to receive both visual and chemical cues from the predator, whereas the predator was unable

to catch the tadpoles. In the no-predator treatment, the cage was left empty. During the experiment, the tadpoles relied on the resources provided in the beginning (leaves, rabbit pellets) and on the algae growing in the tanks. Predators were fed with *R. temporaria* tadpoles (ca. 300 mg) every other day. Each treatment combination was replicated eight times, resulting in a total of 32 experimental units. Treatments were assigned randomly among the tanks.

Body and brain measurements

On day 24 of the experiment, 176 randomly chosen individuals (five from each low density tank and six from each high density tank) were killed with an overdose of MS 222 (tricaine methanesulphonate). Immediately following death, tadpoles were weighed to the nearest 0.01 g with a digital balance, and photographed from dorsal and lateral views, using a digital camera (Nikon D80; Nikon Corp., Tokyo, Japan) equipped with a macro lens (Sigma AF 105 mm f/2.8 EX DG; Sigma Corp, Kanagawa, Japan) in a standardized setup. A millimetre scale was placed in each photograph for scaling. The following measures were later obtained from the digital photographs using tpsDig 1.37 (http://life.bio.sunysb.edu/morph/) software: body length (from the tip of mouth to cloaca), maximum body width, maximum body depth, maximum tail muscle depth, maximum tail depth and tail length (from cloaca to the end of tail). The tadpoles were fixed in 4% formalin – 0.1 M phosphate-buffered saline solution for later dissection of the brains.

Tadpole brains were dissected and put into 4% formalin buffered with 0.1 M phosphate-saline solution. We excluded 12 individuals because of dissection failure, resulting in a total of 164 individuals for brain measurements. After 48 h fixation, dorsal and right lateral views of brains were photographed with a digital camera (Canon EOS 10D; Canon Inc., Tokyo, Japan) connected to a dissecting microscope (Wild M5A; Wild, Heerbrugg, Switzerland). For bilateral structures, only the right-hand side was measured. We could only measure two dimensions for each brain part (length and width of telencephalon, diencephalon and optic tectum, and depth and width of medulla oblongata) because some of the borders of the brain parts could not be identified with accuracy; hence, three dimensional estimations were impossible. Measures were taken from the digital photographs using tpsDig 1.37 software and were defined as the greatest distance enclosed by the given structure. All brains were photographed and measured three times. Repeatability of different brain measurements was high [$R = 0.60–0.95$ (mean = 0.77); $F > 5.60$, $P < 0.001$].

Statistical analyses

To test for the treatment effects on growth in general, we ran General Linear Mixed Models (GLMMs) with total length or body weight as dependent variables, the treatments (predation, density) and their interaction as fixed factors, and replicate (= tank) nested within predation × density as a random factor.

To correct the original body shape variables (body length, body width, body depth, tail length, tail depth, tail muscle depth) for body size, we calculated residuals from regressions between the shape variables and total length. We tested the homogeneity of the slopes of our body shape variables and total length among the different treatment combinations with General Linear Models (GLM ANCOVAs) and found no significant predation × density × total length interaction in any of the cases (all $P > 0.173$), suggesting that the residuals were comparable (see McCoy *et al.*, 2006). To describe body shape with the minimal possible number of independent variables, we ran a Principal Component Analysis (PCA) on the size-corrected variables resulting in two informative PCs (see Results). To test for the treatment effects on body shape, we ran GLMMs with the PC scores as dependent variables, the treatments (predation, density) and their interactions as fixed factors, and replicate nested under predation × density as random factor.

To describe 'brain size' with one variable, we ran a PCA on all variables (length and width of telencephalon, diencephalon and optic tectum, and width and depth of medulla oblongata). Only the first PC was informative, describing brain size (see Results). We followed the same strategy for the separate brain parts, i.e. we ran separate PCAs for the brain parts (telencephalon, diencephalon, optic tectum, medulla oblongata). The first PCs were always informative and described the size of the given structure (see Results). We used these PCs in the subsequent analyses.

To test our hypotheses (treatments effects, relationship with the other treatment-induced morphological changes) in a straightforward manner and to correct for all possible confounding variables, we built complex GLMMs to investigate the patterns in brain development. First, we ran a GLMM with the PC describing brain size as dependent variable, the treatments (predation, density) and their interaction as fixed factors, replicate nested within predation × density as random factor, and total length, body weight and the two shape PCs as covariates. Next, we ran separate GLMMs for the different brain parts, with the PC describing the given brain part as dependent variable, the treatments (predation, density) and their interaction as fixed factors, replicate nested in predation × density as random factor, and total length, body weight, the PC describing brain size and the two shape PCs as covariates. In the case of optic tectum and medulla oblongata, our GLMM indicated the presence of a possible trade-off (see Results); hence, we run an extra GLMM to test this possible trade-off directly. Here, we built a GLMM with the PC describing optic tectum size as dependent variable, the treatments (predation, density) and their interaction as

fixed factors, replicate nested within predation × density as random factor, and the PCs describing brain and medulla oblongata size as covariates. We conducted backward stepwise model selection based on the $P < 0.05$ criteria, but as the model selection did not produce qualitative changes in any of the cases (data not shown), we report results from the original models. SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA) for Windows software package was used for all analyses.

Results

General morphology

We found significant effects of predation risk ($F_{1,\ 27.44} = 8.83$, $P = 0.006$), density ($F_{1,\ 27.44} = 6.45$, $P = 0.017$), and also a predation × density interaction ($F_{1,\ 27.44} = 4.94$, $P = 0.035$; Fig. 1a) on total length. The GLMM on body weight revealed a similar trend, but it was not significant (predation: $F_{1,\ 27.38} = 3.97$, $P = 0.056$; density: $F_{1,\ 27.38} = 1.31$, $P = 0.26$; predation × density: $F_{1,\ 27.38} = 3.87$, $P = 0.059$; Fig. 1b). The replicate effect was nonsignificant in both cases ($Z < 1.75$, $P > 0.08$). Tadpoles were significantly longer and tended to be heavier in the absence of predation risk at low density than in any other treatment combination.

The PCA on the size-corrected shape variables revealed two PCs with eigenvectors > 1, which together accounted for 83.27% of the total variance. Both PCs were biologically meaningful, PC1 (60.68% of total variance) described a gradient from relatively small-bodied and long-tailed tadpoles towards relatively large-bodied and short-tailed tadpoles, whereas PC2 (22.59% of total variance) described a gradient from tadpoles with low tails and tail muscles towards tadpoles with high tails and tail muscles (Appendix S1).

The GLMM on the first shape PC revealed a strong density effect ($F_{1,\ 28.61} = 47.62$, $P < 0.001$; Fig. 1c) without any effect of predation risk (predation: $F_{1,\ 28.61} = 0.12$, $P = 0.91$; predation × density: $F_{1,\ 28.61} = 0.81$, $P = 0.38$; Fig. 1c). The replicate effect was nonsignificant ($Z = 0.86$, $P = 0.39$). Tadpoles at low density had relatively longer tails and smaller bodies than tadpoles at high density. The GLMM on the second shape PC revealed significant density and predation effects with a marginally significant interaction term (predation: $F_{1,\ 22.62} = 12.26$, $P = 0.002$; density: $F_{1,\ 22.62} = 24.93$, $P < 0.001$; predation × density: $F_{1,\ 22.62} = 3.87$, $P = 0.086$; Fig. 1d). The replicate effect was nonsignificant ($Z = 0.92$, $P = 0.35$). Tadpoles at low density or under predation risk had deeper tails and deeper tail muscles than at high density or in the absence of predation risk. The marginally significant interaction term suggests that predation risk had a stronger effect at low tadpole density than at high tadpole density (Fig. 1d).

Brain morphology

The PCA on all brain variables retrieved only one PC with eigenvector > 1 accounting for 70.26% of the total variance. This PC was strongly and positively related to all original variables (factor loadings from 0.71 to 0.93); hence, we treated this PC as describing overall brain size.

The GLMM on the overall brain size revealed a density-dependent effect of predation risk and a positive correlation with shape PC2 (predation: $F_{1,\ 34.53} = 1.41$, $P = 0.24$; density: $F_{1,\ 60.24} = 5.51$, $P = 0.022$; preda-

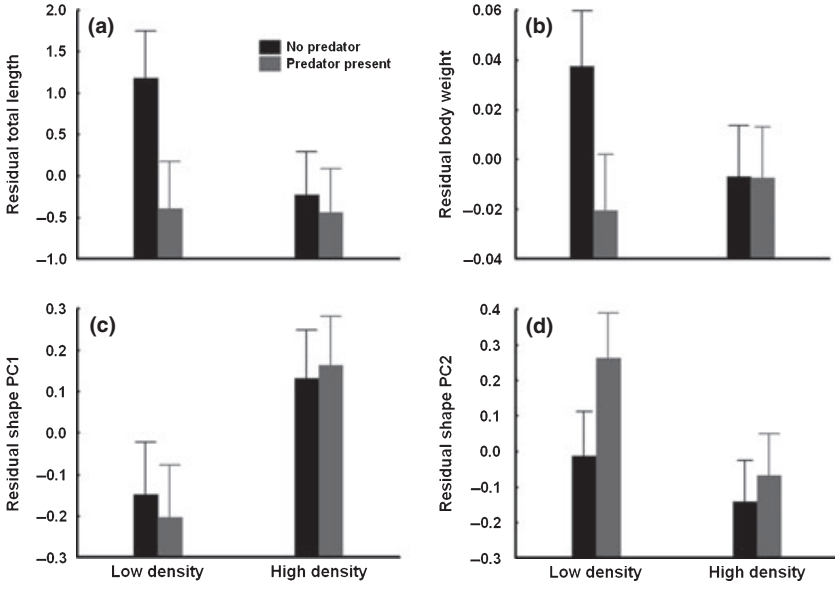

Fig. 1 The effects of density and perceived predation risk on growth and general morphology in *Rana temporaria* tadpoles. (a) length, (b) weight, (c) principal component describing a shape gradient from small-bodied and long-tailed tadpoles towards large-bodied but short-tailed tadpoles, (d) principal component describing a shape gradient from low-tailed and -tail muscled tadpoles towards tadpoles with high tails and tail muscles. Means ± SE are shown. Residuals are calculated from General Linear Mixed Models without the factors density and predation.

tion × density: $F_{1,\ 29.88} = 5.85$, $P = 0.022$; total length: $F_{1,\ 149.99} = 14.93$, $P < 0.001$; body weight: $F_{1,\ 149.72} = 2.42$, $P = 0.12$; shape PC1: $F_{1,\ 153.47} = 0.07$, $P = 0.80$; shape PC2: $F_{1,\ 153.17} = 7.878$, $P = 0.006$). The replicate effect was nonsignificant ($Z = 1.08$, $P = 0.28$). Tadpoles at low density under predation risk developed relatively smaller brains than tadpoles under other treatment combinations (Fig. 2a). Tadpoles having deeper tails and deeper tail muscles had also relatively larger brains (Appendix S2a).

The four PCAs on the different brain parts revealed similar patterns: the first PCs were always strongly and positively related to the original variables (two per brain part; factor loadings from 0.82 to 0.97); hence, we treated them as good size proxies for the given brain part.

We found a significant density effect and a marginally significant predation × density interaction on relative optic tectum size (Table 1). Tadpoles at higher density had relatively larger optic tecta. This effect appeared to be a result of the strong effect of density in the absence of predation risk (Fig. 2b). We also found a significant density effect on the medulla oblongata (Table 1): tadpoles at higher density had relatively smaller medulla oblongata (Fig. 2c). There were no treatment effects on the telencephalon or diencephalon (Table 1). The GLMMs also showed a (i) significant positive correlation with shape PC1 in the medulla oblongata, (ii) significant positive correlation with shape PC2 in the optic tectum and (iii) a marginally significant negative correlation between diencephalon size and shape PC2 (Table 1, Appendix S2b–d). The GLMM testing for direct correlation between the size of optic tectum and medulla oblongata revealed a significant negative relationship

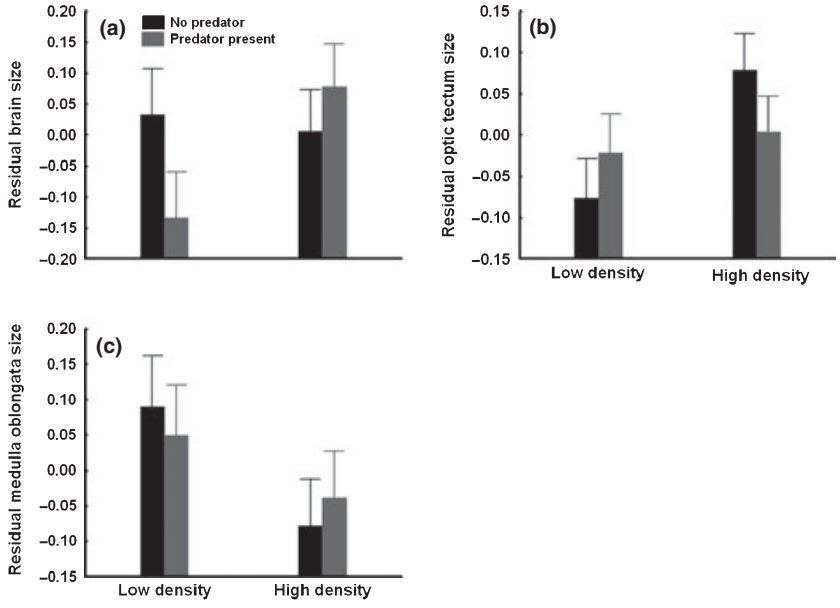

Fig. 2 The effects of density and perceived predation risk on brain development in *Rana temporaria* tadpoles. (a) brain size, (b) optic tectum size, (c) medulla oblongata size. Means ± SE are shown. Residuals are calculated from General Linear Mixed Models without the factors density and predation.

Table 1 Results of the General Linear Mixed Models on the different brain parts.

	Telencephalon		Diencephalon		Optic tectum		Medulla oblongata	
Effect	d.f.	*F*	d.f.	*F*	d.f.	*F*	d.f.	*F*
Predation	1, 32.89	0.23	1, 31.47	1.21	1, 34.63	0.35	1, 36.02	0.71
Density	1, 59.81	0.26	1, 58.01	1.17	1, 61.94	11.05***	1, 63.92	8.75***
Predation × density	1, 29.57	2.72	1, 28.04	0.10	1, 31.29	3.02*	1, 32.55	0.21
Brain size	1, 152.74	263.29****	1, 150.63	130.42****	1, 152.98	292.78****	1, 152.95	158.06****
Total length	1, 149.61	< 0.01	1, 151.51	0.28	1, 148.98	0.93	1, 149.39	< 0.01
Body weight	1, 150.10	1.32	1, 151.85	0.02	1, 149.49	0.04	1, 149.86	0.97
Shape PC1	1, 152.88	1.21	1, 152.73	0.11	1, 152.59	0.25	1, 152.70	4.45**
Shape PC2	1, 150.83	0.39	1, 147.69	3.81*	1, 151.70	5.11**	1, 151.60	0.83

The replicate effects were always nonsignificant ($Z < 1.00$; $P > 0.32$). Shape PC1 describes a gradient from small-bodied and long-tailed tadpoles towards large-bodied but short-tailed tadpoles, whereas shape PC2 describes a shape gradient from low-tailed and -tail muscled tadpoles towards tadpoles with high tails and tail muscles.
*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$, ****$P < 0.001$.

between these traits ($F_{1,\ 155.87} = 30.47$, $P < 0.001$; data not shown).

Discussion

The most salient finding of this study is the influence of ecological interactions on the relative size of the brain and certain brain parts in larval *R. temporaria*, with both density and predation being important factors that shape brain development. In addition, we found that (i) both the presence of predators and high density had a negative effect on growth, (ii) tadpoles raised at high density had relatively larger bodies and shorter tails when compared to those raised at low density and (iii) tadpoles had deeper tails and tail muscles under the predation risk at low density than in the other treatment combinations. Because these results on induced changes in morphology are in accordance with previous studies on tadpoles (McCollum & Van Buskirk, 1996; Van Buskirk & Relyea, 1998; Relyea, 2002; Laurila *et al.*, 2004; Teplitsky & Laurila, 2007), we believe that our results on brain plasticity might also be applied to amphibian larvae in general. Previous studies have found increased survival of induced tadpoles in the presence of lethal predators, which has been linked with the induced beneficial morphology and behaviour (e.g. McCollum & Van Buskirk, 1996; Van Buskirk & Relyea, 1998; Laurila *et al.*, 2006). Similarly, the plastic changes induced by competitors are considered adaptive (Relyea, 2002; Relyea & Auld, 2004, 2005). Our results suggest that the benefits of competitor and predator-induced morphological plasticity are linked with altered neural capacity. Later, we will discuss the implications of this finding while keeping in mind that the demonstration of the potential adaptive value of tadpole brain plasticity has to await for further studies.

We found that predation risk and high intraspecific density that was likely to result in high competition both induced phenotypic plasticity in relative brain size of larval *R. temporaria*. Tadpoles developed relatively smaller brains when they perceived visual and chemical stimuli from a predator but only at low density. Because the brain is energetically the most expensive organ (Aiello & Wheeler, 1995), our results could be explained in terms of energy availability and its impact on brain development (e.g. Taylor & van Schaik, 2007). We suggest that predation risk might be more readily perceived as high at low tadpole density but lower (because of dilution effect) at high density; hence, the presence of a predator might increase risk aversion (manifesting as lowered activity and energy intake) only at low tadpole densities. Perceived predation risk can result in energy deficit not only by lowered activity, as physiological stress responses may also lead to less energy available for development (Stoks *et al.*, 2005; Steiner, 2007; Slos & Stoks, 2008). Another effect, namely that increased competition at high density might make tadpoles more risk-taking, is also conceivable. It has been previously shown that a predation threat affects the activity of tadpoles negatively (Skelly & Werner, 1990; Laurila *et al.*, 1998; Teplitsky & Laurila, 2007), which has been suggested to result in reduced food intake (Werner & Anholt, 1993), and therefore might impose an energetic constraint on brain development.

Seemingly, density alone (i.e. different levels of intraspecific competition) did not pose an energetic challenge that could constrain brain development. As competition may reduce individual food intake at high densities (Anholt & Werner, 1996; Teplitsky & Laurila, 2007), we expected to find negative effects of density on relative brain size. However, this effect was not observed, suggesting that tadpoles at high density did not trade-off relative brain size for increased relative investments into other structures or activities, despite the general growth deficit in this treatment. It has been shown that physical activity directly influences brain size by increasing neurogenesis and decreasing neuronal degradation (Cotman & Berchtold, 2002; Catlow *et al.*, 2009); hence, the level of physical activity per se could be reflected on brain size. Accordingly, more active tadpoles living in the absence of predation and/or under high intraspecific competition (Skelly & Werner, 1990; Anholt & Werner, 1996; Laurila *et al.*, 1998; Teplitsky & Laurila, 2007) might develop relatively bigger brains compared to less active tadpoles living at low density under high individual predation risk. This expectation is also supported by our data.

We found that relative optic tectum (the main centre for vision) size was significantly larger at high than at low tadpole densities. It seems feasible to suggest that increased competition for food at high density imposes higher demands on optic tectum, inducing its growth. Furthermore, vision is involved in communication and perception of social environment (Hoff *et al.*, 1999), and these needs can also be expected to be more pronounced at high densities. A nonsignificant trend ($P < 0.1$) for an interaction between predation and density was found, presuming that predation might have a positive effect on optic tectum size at low density, whereas the opposite trend was observed at high density. Although olfactory cues are especially important in predator detection in tadpoles (Kats & Dill, 1998; Schoeppner & Relyea, 2005), vision also plays some role in both predator detection and localization (e.g. Semlitsch & Reyer, 1992). Hence, the enlargement of the optic tectum under predation risk at low density (with high individual risk) can be expected. The decreased optic tectum development under predation risk at high density is less straightforward to explain. However, trade-offs among different brain parts can occur (Barton *et al.*, 1995; Barton & Harvey, 2000), and size of optic tectum in the predatory treatment at high density could be traded-off with some other – yet unidentified – brain structure required for anti-predator behaviour. An alternative explanation could be that at

high density (where large optic tectum is favoured), predation imposed an energetic constraint, so opposite to the situation at low density, predation constrained maximal optic tectum development.

In contrast to the optic tectum, we found that the size of the medulla oblongata was significantly larger at low density when compared to high density treatment. The medulla oblongata is involved in regulation of respiratory, auditory and lateral line system functions in tadpoles (Torgerson *et al.*, 2001; McCormick, 1999; Jacoby & Rubunson, 1983). Previous studies in brain development have demonstrated that those parts of the brain likely to be important in a particular context develop more than those of less importance (e.g. Kihslinger & Nevitt, 2006; Lisney *et al.*, 2007). It has also been shown from an evolutionary perspective that changes in demand alter the number and size of component elements, making the relative size of different brain parts a reliable predictor of their importance for the organism in question (Kotrschal *et al.*, 1998). We assume that under low intraspecific competition environments where demands for good vision are lower than in high competition environments, other sensory systems such as lateral line and vestibular become more important. As a consequence, tadpoles reared at low densities develop smaller optic tectum and larger medulla oblongata compared to tadpoles reared at high densities. Trade-offs among different brain parts have been shown to occur in different taxa at both evolutionary and ontogenetic levels (e.g. Barton *et al.*, 1995; Barton & Harvey, 2000; Gonda *et al.*, 2009). Hence, an alternative explanation could be that the medulla oblongata is in a trade-off relationship with the optic tectum, so when the relative size of optic tectum became enhanced for higher competitive ability, the medulla oblongata became smaller because of energetic or developmental constraints.

We also assessed the possible relationships between treatment-induced morphological (body shape) and brain differences to evaluate if an increased investment into morphology (e.g. into tail muscles for better locomotive performance) was related to the enhancement of certain brain structures. Interestingly, we found that optic tectum increased with increasing tail and tail muscle depth, and medulla oblongata increased with increasing body size and decreasing tail length. Although these trends might seem to contradict what was found in the analyses of treatment effects on body shape and brain morphology, this is not the case. The correlations between body shape and brain structures discussed here are corrected for the treatment effects and describe treatment-independent relationships. The finding that tadpoles with deeper tail and tail muscle had larger optic tectum suggests that stronger predator-induced morphology is connected to enhanced visual abilities. Tadpoles with relatively larger bodies and shorter tails had relatively larger medulla oblongata; this aligns with the contention that the competition-induced phenotype requires more developed lateral line and vestibular sensory systems.

In summary, our results demonstrate that predation risk and high density that was likely to result in high intraspecific competition – two commonplace ecological interactions – influence brain development of larval common frogs. First, we found that tadpoles developed relatively small brains when reared in a combined treatment of predator risk and low tadpole density, probably as a result of the constrained energy intake because of a risk-averse behavioural strategy (low activity, limited foraging) adopted under high per capita predation risk. Second, we found opposite patterns in relative optic tectum and medulla oblongata size: tadpoles had relatively larger optic tectum and smaller medulla oblongata at high tadpole density. This might be a result of either opposing demands of these brain parts under different situations, or a trade-off between the two structures. It is noteworthy that the density effect on optic tectum was mainly driven by the differences observed in the absence of predation; predation might have opposing effects on the optic tectum at different densities. Our results also raise an interesting question: does larval experience during the aquatic phase affect the brain structure and neural abilities of metamorphosed, terrestrial frogs? Future research is needed to study the adaptive value of brain plasticity, energetic constraints and trade-offs involved in brain development, as well as potential carry-over effects in brain architecture to later life stages.

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References

Adar, E., Lotem, A. & Barnea, A. 2008. The effect of social environment on singing behavior in the zebra finch (*Taeniopygia guttata*) and its implication for neuronal recruitment. *Behav. Brain Res.* **187**: 178–184.

Aiello, L.C. & Wheeler, P. 1995. The expensive-tissue hypothesis. *Curr. Anthropol.* **36**: 199–221.

Anholt, B.R. & Werner, E.E. 1996. Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. *Ecology* **77**: 157–169.

Barton, R.A. & Harvey, P.H. 2000. Mosaic evolution of brain structure in mammals. *Nature* **405**: 1055–1058.

Barton, R.A., Purvis, A. & Harvey, P.H. 1995. Evolutionary radiation of visual and olfactory brain systems in primates, bats and insectivores. *Phil. Trans. Biol. Sci.* **348**: 381–392.

Callahan, H.S., Maughan, H. & Steiner, U.K. 2008. Phenotypic plasticity, costs of phenotypes, and costs of plasticity: toward an integrative view. *Ann. N.Y. Acad. Sci.* **1133**: 44–66.

Catlow, B.J., Rowe, A.R., Clearwater, C.R., Mamcarz, M., Arendash, G.W. & Sanchez-Ramos, J. 2009. Effects of environmental enrichment and physical activity on neurogenesis in transgenic PS1/APP mice. *Brain Res.* **1256**: 173–179.

Cotman, C.W. & Berchtold, N.C. 2002. Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci.* **25**: 295–301.

Font, E., Desfilis, E., Pérez-Cañellas, M.M. & Garcıa-Verdugo, J.M. 2001. Neurogenesis and neuronal regeneration in the adult reptilian brain. *Brain Behav. Evol.* **58**: 276–295.

Fowler, D.C., Liu, Y., Ouimet, C. & Wang, Z. 2002. The effects of social environment on adult neurogenesis in the female prairie vole. *J. Neurobiol.* **51**: 115–128.

Gonda, A., Herczeg, G. & Merilä, J. 2009. Habitat-dependent and -independent plastic responses to social environment in the nine-spined stickleback (*Pungitius pungitius*) brain. *Proc. R. Soc. B* **276**: 2085–2092.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**: 183–190.

Gould, E., Tanapat, P., Hastings, N.B. & Shors, T.J. 1999. Neurogenesis in adulthood: a possible role in learning. *Trends Cogn. Sci.* **3**: 186–192.

Hoff, K.S., Blaustein, A.R., McDiarmid, R.W. & Altig, R. 1999. Behaviour: interactions and their consequences. In: *Tadpoles: The Biology of Anuran Larvae* (R.W. McDiarmid & R. Altig, eds), pp. 215–239. University of Chicago, Chicago.

Jacoby, J. & Rubunson, K. 1983. The acoustic and lateral line nuclei are distinct in the premetamorphic frog, *Rana catesbeiana*. *J. Comp. Neurol.* **216**: 152–161.

Kats, L.B. & Dill, L.M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**: 361–394.

Kempermann, G., Kuhn, H.G. & Gage, F.H. 1997. More hippocampal neurons in adult mice living in enriched environment. *Nature* **386**: 493–495.

Kihslinger, R.L. & Nevitt, G.A. 2006. Early rearing environment impacts cerebellar growth in juvenile salmon. *J. Exp. Biol.* **209**: 504–509.

Kihslinger, R.L., Lema, S.C. & Nevitt, G.A. 2006. Environmental rearing conditions produce forebrain differences in wild Chinook salmon *Oncorhynchus tshawytscha*. *Comp. Biochem. Physiol. A* **145**: 145–151.

Köhler, M. & Moyà-Solà, S. 2004. Reduction of brain and sense organs in the fossil insular bovid Myotragus. *Brain Behav. Evol.* **63**: 125–140.

Kotrschal, K., Van Staaden, M.J. & Huber, R. 1998. Fish brains: evolution and environmental relationships. *Rev. Fish Biol. Fish.* **8**: 373–408.

Laurila, A. 1998. Breeding habitat selection and larval performance of two anurans in freshwater rock-pools. *Ecography* **21**: 484–494.

Laurila, A., Kujasalo, J. & Ranta, E. 1998. Predator-induced changes in life-history in two anuran tadpoles: effects of predator diet. *Oikos* **83**: 307–317.

Laurila, A., Jarvi-Laturi, M., Pakkasmaa, S. & Merilä, J. 2004. Temporal variation in predator risk: stage-dependency, graded responses and fitness costs in tadpole antipredator defences. *Oikos* **107**: 90–99.

Laurila, A., Pakkasmaa, S. & Merilä, J. 2006. Population divergence in growth rate and antipredator defences in *Rana arvalis*. *Oecologia* **147**: 585–595.

Lisney, T.J., Bennett, M.B. & Collin, S.P. 2007. Volumetric analysis of brain areas indicates a shift in sensory orientation during development in the deep-sea grenadier *Coryphaenoides armatus*. *Raffles Bull. Zool.* **14**: 7–15.

McCollum, S.A. & Van Buskirk, J. 1996. Costs and benefits of a predator induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* **50**: 583–593.

McCormick, C.A. 1999. Comparative Hearing: Fish and Amphibians. In: *Handbook of Auditory Research* (A.N. Popper & R.R. Fay, eds), pp. 155–217. Springer-Verlag, New York.

McCoy, M.W., Bolker, B.M., Osenberg, C.W., Miner, B.G. & Vonesh, J.R. 2006. Size correction: comparing morphological traits among populations and environments. *Oecologia* **148**: 547–554.

Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. & Relyea, R.A. 2005. Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.* **20**: 685–692.

Nottebohm, F. 1981. A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. *Science* **214**: 1368–1370.

Piersma, T. & Drent, J. 2003. Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* **18**: 228–233.

Relyea, R.A. 2002. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecol. Monogr.* **72**: 523–540.

Relyea, R.A. & Auld, J.R. 2004. Having the guts to compete: how intestinal plasticity explains costs of inducible defences. *Ecol. Lett.* **7**: 869–875.

Relyea, R.A. & Auld, J.R. 2005. Predator- and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. *Ecology* **86**: 1723–1729.

Rhode, J.S., van Praag, H., Jeffrey, S., Girard, I., Mitchell, G.S., Garland, T. & Gage, F.H. 2003. Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. *Behav. Neurosci.* **117**: 1006–1016.

Schlichting, C.D. & Pigliucci, M. 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer, Sunderland, MA.

Schoeppner, N.M. & Relyea, R.A. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecol. Lett.* **8**: 505–512.

Semlitsch, R.D. & Reyer, H.U. 1992. Modification of antipredator behavior in tadpoles by environmental conditions. *J. Anim. Ecol.* **61**: 353–360.

Skelly, D.K. & Werner, E.E. 1990. Behavioral and life-historical responses of larval American toads to an odonate predator. *Ecology* **71**: 2313–2322.

Slos, S. & Stoks, R. 2008. Predation induces stress proteins and reduces antioxidant defense. *Funct. Ecol.* **22**: 637–642.

Sorensen, C., Overly, O., Summers, C.H. & Nilson, G.E. 2007. Social regulation of neurogenesis in teleosts. *Brain Behav. Evol.* **70**: 239.

Steiner, U.K. 2007. Linking antipredator behaviour, ingestion, gut evacuation and costs of predator-induced responses to tadpoles. *Anim. Behav.* **74**: 1473–1479.

Stoks, R., de Block, M., van de Meutter, F. & Johansson, F. 2005. Predation cost of rapid growth: behavioural coupling and physiological decoupling. *J. Anim. Ecol.* **74**: 708–715.

Taylor, A.B. & van Schaik, C.P. 2007. Variation in brain size and ecology in Pongo. *J. Hum. Evol.* **52**: 59–71.

Teplitsky, C. & Laurila, A. 2007. Flexible defense strategies: competition modifies investment in behavioral vs. morphological defenses. *Ecology* **88**: 1641–1646.

Torgerson, C.S., Gdovin, M.J. & Remmers, J.E. 2001. Sites of respiratory rhythmogenesis during development in the tadpole. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**: 913–920.

Tramontin, A.D. & Brenowitz, E.A. 2000. Seasonal plasticity in adult brain. *Trends Neurosci.* **23**: 251–258.

Van Buskirk, J. 2001. Specific induced responses to different predator species in anuran larvae. *J. Evol. Biol.* **14**: 482–489.

Van Buskirk, J. & Relyea, R.A. 1998. Selection for phenotypic plasticity in *Rana sylvatica* tadpoles. *Biol. J. Linn. Soc.* **65**: 301–328.

Van Praag, H., Kempermann, G. & Cage, F.H. 2000. Neural consequences of environmental enrichment. *Nat. Rev.* **1**: 191–198.

Werner, E.E. & Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality-rates mediated by foraging activity. *Am. Nat.* **142**: 242–272.

Zupanc, G.K.H. 2001. Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. *Brain Behav. Evol.* **58**: 250–275.

Supporting information

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

Appendix S1 Factor loadings from the principal component analysis on the body shape variables.

Appendix S2 Correlations between certain brain structures and the level of investments into treatment-induced body shape change.

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