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Rapid plastic changes in brain morphology in response to acute changes in predation pressure in juvenile Atlantic salmon (*Salmo salar*) and northern redbelly dace (*Phoxinus eos*)

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Abstract: Teleosts exhibit inter- and intra-specific variation in the size and shape of their brains. Interpopulation differences in gross brain morphology among numerous teleost fish species have been observed and have been partially attributed to plastic changes in response to their environment, including predation. These differences manifest themselves macroscopically, potentially because teleosts retain the capacity for active neuroproliferation into adulthood. Building on previous work, showing chronic exposure to predation can affect brain morphology, we sought to determine whether these differences manifest themselves on a time scale shown to induce phenotypically plastic behavioural changes. In separate trials, we held northern redbelly dace (*Phoxinus eos* (Cope, 1861) = *Chrosomus eos* Cope, 1861) and juvenile Atlantic salmon (*Salmo salar* Linnaeus, 1758) in semi-natural conditions and exposed them to conspecific skin extract as a proxy for predation risk over 2 weeks. After exposure, their brains were excised, photographed, and analyzed for size (multivariate ANOVA) and shape (Procrustes ANOVA). Despite their brief exposure to simulated predation pressure, subjects from both species developed significantly different brain morphologies. Compared with controls, the Atlantic salmon exhibited a different brain shape and smaller optic tecta, whereas the northern redbelly dace had larger brains with more developed olfactory bulbs and optic tecta. Our results highlight the rapidity with which external environment can alter patterns of growth in the brain.

Key words: predation risk, gross brain morphology, plasticity, teleost, juvenile *Salmo salar*, *Phoxinus eos*, *Chrosomus eos*.

Résumé : Les téléostéens présentent des variations inter- et intra-spécifiques de la taille et de la forme du cerveau. Des variations de la morphologie grossière du cerveau entre populations pour de nombreuses espèces de poissons téléostéens ont été observées et en partie attribuées à des changements plastiques des réactions au milieu, incluant la prédation. Ces différences ont des manifestations macroscopiques, possiblement en raison du fait que les téléostéens adultes conservent la capacité de neuroprolifération active. En partant de travaux antérieurs qui montrent que l'exposition chronique à la prédation peut avoir une incidence sur la morphologie du cerveau, nous avons tenté de déterminer si ces différences se manifestent à une échelle temporelle dont il est démontré qu'elle induit des changements comportementaux plastiques du point de vue phénotypique. Dans des essais distincts, nous avons maintenu des ventres rouges du Nord (*Phoxinus eos* (Cope, 1861) = *Chrosomus eos* Cope, 1861) et des saumons atlantiques (*Salmo salar* Linnaeus, 1758) juvéniles dans des conditions semi-naturelles et les avons exposés pendant deux semaines à de l'extrait de peau de congénères simulant un risque de prédation. Après l'exposition, leurs cerveaux ont été excisés, photographiés et analysés pour en déterminer la taille (analyse de la variance multivariée) et la forme (analyse de la variance procustéene). Malgré la courte exposition à une pression de prédation simulée, les cerveaux des sujets des deux espèces ont subi des changements morphologiques significatifs. Comparés aux témoins, les saumons atlantiques présentaient une forme du cerveau différente et des tectums optiques plus petits, alors que les ventres rouges du Nord avaient des cerveaux plus grands présentant des bulbes olfactifs et tectums optiques plus développés. Nos résultats font ressortir la rapidité avec laquelle le milieu externe peut modifier des motifs de croissance dans le cerveau. [Traduit par la Rédaction]

Mots-clés : risque de prédation, morphologie grossière du cerveau, plasticité, téléostéen, *Salmo salar* juvénile, *Phoxinus eos*, *Chrosomus eos*.

Introduction

Studies have demonstrated high degrees of variability in the brain morphology of teleost fishes, both within and between species (Kotrschal et al. 1998). Within species, variation in brain morphology between populations is often attributable to differing local ecological parameters (Cadwallader 1975; Ebbesson and Braithwaite 2012). For example, cave-dwelling populations of the shortfin molly (*Poecilia mexicana* Steindachner, 1863) have severely reduced optic tecta compared with surface populations (Eifert et al. 2015). In the teleost brain, cell proliferation and neurogenesis occurs continuously (Zupanc 2006; Kaslin et al. 2008) and may account for a high degree of adaptive phenotypic plasticity in brain morphology (Gonda et al. 2013; Eifert et al. 2015; Olivera-Pasilio et al. 2017). This plasticity in brain morphology is often measured as differences in the size of distinct brain regions relative to whole brain size or body size (Gonda et al. 2013). Additionally, as a result of neurogenesis, subregions also exhibit considerable variation (Boulanger-Weill and Sumbre 2019), resulting in changes in shape independent of relative size. As a result, the impacts of ecological parameters on brain morphology may be measured as differences in relative size of specific regions and (or) overall shape.

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The dorsal surface of a teleost brain has four visually distinct regions: the olfactory bulbs, telencephalic pallium, optic tectum, and cerebellum. The metabolic cost of neural tissue is high, and the expense of producing and maintaining it is believed to impose strong constraints on brain size (Aiello and Wheeler 1995; Tsuboi et al. 2015). Consequently, variation in the relative size of brains and their structures between populations of a species reflects energetic investment which correlates with the utility of that region for a population (Kaslin et al. 2008; Kotrschal et al. 2015, 2017).

Recent studies have identified an array of factors that may influence the relative size of these regions. For example, under hatchery conditions, high population density in Atlantic salmon (*Salmo salar* Linnaeus, 1758) results in larger telencephalons and cerebella (Näslund et al. 2017), while captive rearing produces larger optic tecta in juvenile coho salmon (*Oncorhynchus kisutch* (Walbaum, 1792)) (Kotrschal et al. 2012). Ninespine sticklebacks (*Pungitius pungitius* (Linnaeus, 1758)) raised in a social environment have been shown to invest more in their optic tecta and less in olfaction (Gonda et al. 2009*b*). These studies demonstrate the plasticity of teleost brains with respect to their environment.

Perceived predation risk can likewise shape brain morphology. Laboratory experiments with fish raised under elevated predation, such as freshwater ninespine sticklebacks, male guppies (*Poecilia reticulata* Peters, 1859), and Panamanian bishops (*Brachyrhaphis episcopi* (Steindachner, 1878)), have shown differential investment in overall brain size and (or) specific regions, including relatively larger olfactory bulbs and optic tecta, smaller hypothalami, and altered patterns of brain lateralization (Brown et al. 2004; Gonda et al. 2012; Reddon et al. 2018). Notably, these patterns can vary across environments (e.g., marine vs. freshwater) and contexts (e.g., the hunting strategy of local predators) both within and between species and this may result from local adaptation (Gonda et al. 2012; Eifert et al. 2015; Kotrschal et al. 2017; Samuk et al. 2018).

These results are echoed by comparisons between populations with differing degrees of predation in the wild across several species, including ninespine stickleback and guppies (Gonda et al. 2012; Kotrschal et al. 2017; Reddon et al. 2018). Additionally, recent work with guppies has found complex associations between mass of brain regions and predation pressure (Kotrschal et al. 2017; Reddon et al. 2018). Male guppies from high-predation streams and those raised in the laboratory with cues of elevated predation risk invest more in neural tissue than conspecifics from low-predation conditions (Reddon et al. 2018). In female guppies, exposure to predatory prawns (species of the genus *Macrobrachium* Bate, 1868) was correlated with larger brains and increased telencephalic investment, whereas the biomass of the blue acara cichlid (*Andinoacara pulcher* (Gill, 1858)) was associated with reduced olfactory bulb and hypothalamus size (Kotrschal et al. 2017).

Typically, this type of study has been conducted using either wild-caught or laboratory (hatchery) strains of prey exposed to some degree of chronic predation (Gonda et al. 2011; Handelsman et al. 2013; Kotrschal et al. 2017). The related laboratory studies have employed long periods of conditioning with levels of perceived risk elevated for one or more months (Gonda et al. 2009*a*, 2012, 2013; Näslund et al. 2017; Reddon et al. 2018). In both the wild and the laboratory, subjects exposed to elevated predation for extended periods exhibited distinct brain morphologies. However, predation can be highly variable, both within an animal's range and across its lifetime (Schauber et al. 2009); predation's propensity for change combined with its lethal nature is expected to exert a strong selective pressure on the phenotypes of prey (Lima and Dill 1990; Relyea 2002).

Phenotypic plasticity in life history, morphology, and behaviour enables prey species to reduce their risk of consumption when confronted with an elevation in perceived predation pressure (Benard 2004). Of the three, behaviour is often the most plastic and least costly (Snell-Rood 2013; Murren et al. 2015). Individuals exposed to an increase in risk display antipredator behaviour immediately upon exposure and develop distinct behavioural phenotypes in a matter of days (Ferrari 2014; Brown et al. 2015; Joyce et al. 2016). Teleost behaviour has been shown to correlate with brain morphology (Schnitzlein 1964; Ito et al. 2007; Burns and Rodd 2008; Gonda et al. 2009*b*).

Here, we set out to determine if differences in brain morphology are detectable on a time scale commensurate with the behavioural plasticity previously observed in fish. We used conspecific chemical alarm cues, which are released from damaged skin and serve as a reliable indicator of an injured conspecific, to manipulate perceived predation risk (Mirza and Chivers 2003; Wisenden et al. 2010). We used two species of teleost fish known to employ such cues: juvenile Atlantic salmon and northern redbelly dace (*Phoxinus eos* (Cope, 1861) = *Chrosomus eos* Cope, 1861) (Dupuch et al. 2004; Brown et al. 2011).

Our choice of a 2-week time frame for these experiments was informed by observations in several related fields of study. First, a reasonable upper limit for the time required for detectable plastic changes in the brain was established. We based this on the time required to regenerate an olfactory bulb damaged by lesioning, approximately 3 weeks in zebrafish (*Danio rerio* (Hamilton, 1822)), or complete removal, 4 weeks in goldfish (*Carassius auratus* (Linnaeus, 1758)) (Zippel et al. 1993; Paskin et al. 2011). Second, a lower limit was suggested by work in threespine sticklebacks (*Gasterosteus aculeatus* Linnaeus, 1758), which after 6 days of exposure to predator cues had significant transcriptomic changes in their brain tissue (Sanogo et al. 2011).

Finally, as mentioned, often the first outwardly observable changes in an organism in response to elevated predation are behavioural (Mirza and Chivers 2003; Wisenden et al. 2010; Elvidge et al. 2014). Evidence from studies conducted under natural conditions, such as with the Panamanian bishop, suggest that predation pressure selects for bolder behavioural phenotypes (Brown et al. 2005; Archard and Braithwaite 2011). Work in threespine sticklebacks suggests that plastic changes in personality can occur rapidly, with increased boldness being detected 1 week following exposure to a live salmonid predator (Bell and Sih 2007). Based on these combined observations, we hypothesized that macroscopically detectable differences in the relative size of brain regions and (or) overall brain shape, in response to additional predation risk, may be expected to manifest within 14 days.

Materials and methods

Stimulus preparation

We collected alarm cues from 16 Atlantic salmon (experiment 1) and 27 northern redbelly dace (experiment 2). Alarm-cue donors were euthanized by cervical dislocation in accordance with Concordia University's Animal Research Ethics protocol (AREC 30000255). We removed their skin by dissection and recorded the dimensions of the skin fillets. The fillets were homogenized with distilled water, filtered through polyester fiber, and diluted to a final concentration of 0.15 cm^2/mL. The resulting alarm cue was divided into 10 mL aliquots in plastic bags and frozen at –20 °C until needed.

Collection, preservation, and sample storage

The subjects were euthanized using an overdose of clove oil (prepared in accordance with Concordia University's Animal Research Ethics protocol AREC 30000255), weighed, and photographed. The subjects were individually preserved in 20 mL glass scintillation vials containing a solution of 3.7% paraformaldehyde in phosphate-buffered saline (PBS). Upon transfer to the laboratory, the preservative was replaced with fresh solution and the samples refrigerated at 4 °C.

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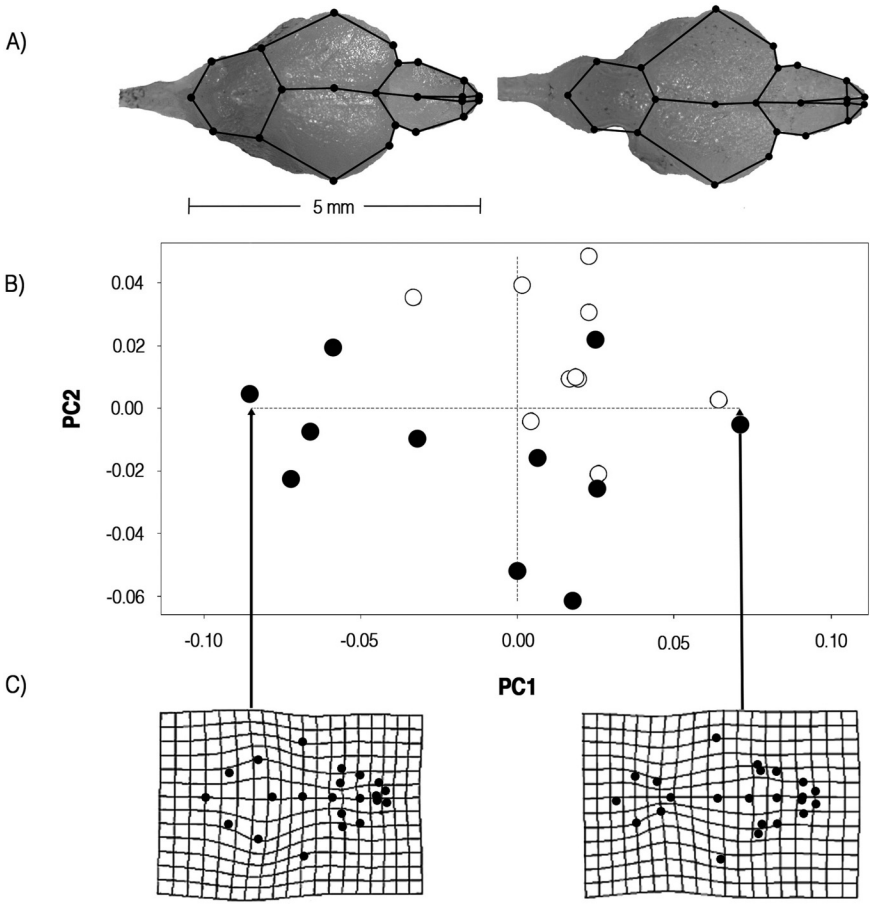
Fig. 1. Procrustes analysis of the juvenile Atlantic salmon (*Salmo salar*) showing (A) the configuration of landmarks and polygons used in the analysis overlaid on two example Atlantic salmon brains, which exhibit the sort of variation observed in (B) a principal components (PC) plot of the distribution of brain shapes by treatment (ambient risk (black circles) and heightened risk (white circles)) and (C) wireframe plots representing the shape configurations, at either end of the PC1 axis (arrows), as deformations (warps) of the mean shape for all specimens.



Sample preparation

Brains were extracted by removing the neurocranium and severing the optic nerves and the spinal cord at the entrance of the vertebral column (Noguera et al. 2015). Once removed, they were placed beside a millimetric grid on high-contrast magenta felt saturated with PBS, which facilitated positioning and prevented them from drying out. The dorsal surface of each brain was photographed using a 3 MP microscope camera affixed to an 8× to 35× binocular dissection scope using a small halogen spot lamp as a light source. Specimens, or portions thereof, damaged during dissection were coded, but only intact specimens were used in morphometric and multivariate size analyses.

Photographic analysis

Our approach to photographic analysis was adapted from Pollen et al. (2007) and modified to also permit Procrustean analysis with the geomorph package in R version 3.03 (Adams et al. 2017), which enabled us to estimate regional area and to analyze an additional aspect of the data (i.e., brain shape) that should reflect differences in relative regional investment. Procrustean analysis allowed regional size or shape variation to be visualized and compared between treatments.

Using ImageJ (Schneider et al. 2012), all images were coded, blind to treatment, and in random order by the addition of landmarks, corresponding to readily identifiable anatomical points, thereby defining each brain region (Fig. 1A). Cross-sectional areas were estimated as the area of the resulting *n*-sided polygon for each region and used as a proxy for regional size. Damaged specimens showing signs of separation between the telencephalon and the optic tectum were excluded from morphometric analysis and encoded with additional landmarks to permit estimation of cross-sectional area when landmarks were no longer shared between otherwise intact regions. Example brains were selected from samples collected in 2016, from the same source populations, and photographed with a 5 MP Leica™ EZ4W HD dissecting scope using incident and oblique LED illumination. The images were isolated from their backgrounds, converted to grayscale, and overlaid with landmarks and polygons using Adobe Photoshop.

Experiment 1: juvenile Atlantic salmon

Fish collection

We obtained hatchery-raised Atlantic salmon from the Miramichi Salmon Conservation Centre and transported them to Catamaran Brook (46°52.7′N, 66°06.0′W), which is a nearby natural salmon habitat with parameters similar to those in the hatchery. Fry (n = 20) were haphazardly distributed into and held in 60 L transparent plastic bins (Sterelite™, 60 cm × 40 cm × 34 cm). We modified the totes cutting three windows (15 cm × 15 cm) in each of the long sides and covered them in 3 mm hardware cloth. The four bins were anchored in the Catamaran Brook to a steel I-beam, filled with a shallow layer of gravel substrate from the stream bed, and separated from one another by 0.5 m and with a water depth of 0.4 m. The windows were perpendicular to the water flow to enable drift fodder to enter for natural feeding. During the experiment, the mean (±SD) current velocity, adjacent to the enclo-

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sures, was 0.33 ± 0.01 m/s and the mean (±SD) water temperature was 20.6 ± 1.8 °C.

Alarm-cue exposure

To manipulate background predation risk, we created two groups: heightened risk (HR group), exposed to 10 mL of salmon alarm cue, and ambient risk (AR group), exposed to stream water, twice per day for 14 days. Ambient risk was selected to reflect the potential for exposure to alarm cue from wild salmon upstream. The cue was released while moving the syringe back and forth along the bin's windows and approximately 5 cm upstream. In an effort to enhance the perceived risk and reduce predictability, the exact timing of the morning and evening treatments was varied. The lids of the bins were opened daily for welfare checks; on three occasions, the elevated risk subjects were exposed with the lids open. Exposure occurred over approximately 14 days with the last treatment occurring 24 h prior to euthanasia.

Morphometric analysis

A statistical analysis of shape variation and covariation of shape with risk was performed in RStudio running the geomorph package (RStudio Team 2015; Adams et al. 2017). First, each data set underwent a generalized Procrustes analysis (GPA) employing the raw landmark coordinates and scaling data obtained with ImageJ. GPA calculates the centroid size (CS) of each brain as the square root of the sum of squared distances from each landmark to the specimen's centroid. GPA also rotates all landmark configurations to a shared coordinate system and unit size and generates shape data as the Procrustes distances between specimens (Adams et al. 2013; Sherratt 2014). The Procrustes distance between two specimens is a measure of the difference between two sets of coordinates following Procrustes superimposition, calculated as the square root of the summed squared distances between the landmarks (Mitteroecker et al. 2013). We evaluated our shape data graphically for outliers by treatment, using the plotOutliers function on the Procrustes aligned coordinates by group, and none were found. Then the data were tested for shape covariation, with centroid size serving as a proxy for brain size, using the procD.allometry function that included a test for homogeneity of slopes; no significant allometric relationships were found and the slopes were homogeneous. The output of the GPA was used to run a Procrustes ANOVA with shape as the dependent variable, risk as a fixed factor, and CS as the covariate, including a risk by the CS interaction term. Procrustes ANOVAs employ a permutation procedure to generate random test statistics for comparison with the original and requires a user-specified number of iterations (Sherratt 2014). To minimize the variance around the reported p values, all analyses were carried out at 5500 permutations (Adams and Anthony 1996). No significant interaction between risk and CS was found in Atlantic salmon or northern redbelly dace ($p > 0.05$) and the interaction was omitted from later analysis. Principal components analysis (PCA) including deformation grids were generated using the geomorph function plotTangentSpace (Adams et al. 2017).

Size analysis

Areas were obtained for each lobe separately and totaled by region: olfactory, telencephalic pallium, optic, and cerebellar. The sum of these areas was total brain area. The allometric relationship of brain with body size was controlled for by $\log_{10}$ transformation of the regional values (Kotrschal et al. 2012). Measures of standard length (SL) and mass (M) were $\log_{10}$-transformed and we computed Fulton's condition factor as $K = 100 \times M \times SL^{-3}$. Our data were analyzed using IBM SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, New York, USA).

The same initial multivariate GLM analysis was used for both species. First, the residuals for all transformed variables were assessed for normality with Shapiro–Wilk W tests ($p > 0.05$). Our model included all four brain regions with risk level as a fixed factor and the $\log_{10}$ of standard length as a covariate. The model was run with the inclusion of a length × risk interaction term; no significant interaction was found ($p > 0.05$) and non-significant interaction terms were excluded from subsequent analysis (Kotrschal et al. 2012). We followed up on significant results from the full factorial model with univariate GLM, initially including enclosure as a random factor and a length × enclosure interaction term. No effect of enclosure was found ($p > 0.05$) and it was excluded from the final model.

Experiment 2: northern redbelly dace

Fish collection

Our subjects were northern redbelly dace, a common and widely distributed bait fish (Stasiak 2006). They were from an isolated population inhabiting a man-made pond (2.5 ha) in Kenyon township, Ontario, Canada, and were collected using baited (white bread) Gee's Improved minnow traps. Upon capture, we identified the northern redbelly dace visually by comparison to reference images (Froese and Pauly 2018; Lyons 2018). They were separated from the bycatch, which consisted primarily of similarly sized finescale dace (*Phoxinus neogaeus* (Cope, 1861) = *Chrosomus neogaeus* (Cope, 1867)) and northern redbelly dace × finescale dace (*P. eos* × *P. neogaeus*) hybrids; the bycatch was subsequently released.

Upon being sorted, we transferred all of the captured northern redbelly dace to a 40 L glass aquarium fitted with an air stone and charcoal filter and held them for 24 h to assess their health and confirm their species prior to distribution. We distributed the northern redbelly dace across 10 containers haphazardly with 10 per container. Two of the containers were randomly selected as wild-caught controls and sacrificed by anesthetic overdose and preserved immediately.

The northern redbelly dace enclosures were 19 L white plastic buckets, which we modified with six 7.6 cm diameter holes (two on the base and four on the sides) covered by 6.5 mm galvanized hardware cloth fastened with steel rivets. These were suspended in the pond at the field site such that the northern redbelly dace occupied approximately 16 L. To deter possible predation and prevent jump outs, we covered them with bird netting. The buckets in each group were spaced 30 cm apart, the distance between the groups was 133 cm, and they were arrayed along the shoreline of a peninsula, projecting into the pond, with their holes oriented to ensure visual isolation between them. The meshed holes provided a combined 270 cm^2 of interface with the environment and permitted the northern redbelly dace to feed on drift fodder passing through the holes. Additional food in the form of tropical fish flake food (Nutrafin Max™, Hagen) was provided once daily at dusk. Excess food and waste exited the enclosure through two mesh-covered holes in the base. The enclosures were held over water 1.5 m deep, and during the experiment, the mean (±SD) noon water temperature was 21.3 ± 1.4 °C.

Alarm-cue exposure

Similar to experiment 1, we employed HR and AR groups; 10 mL of alarm cue or water was introduced into the enclosures twice a day. This was introduced by syringe via a length of airline tubing that led to a fixed release point approximately 5 cm below the water line. The cue was allowed to disperse within the enclosure for 5 min before the enclosures were flushed by pushing water through the enclosures laterally by means of a boat oar. The timing of the treatments and the interval between first and second injections varied from day to day in an intentionally unpredictable fashion, resulting in the only reliably risk-free period being between midnight (0000) and 0600. Exposure occurred over approximately 14 days with the last treatment occurring 36 h prior to euthanasia.

Table 1. Mean (±SE) values for parameters measured for juvenile Atlantic salmon (*Salmo salar*) in heightened vs. ambient risk conditions.

	Ambient risk	Heightened risk	*F*	*p*
Standard length (mm)	32.20 ± 0.86	32.47 ± 0.54	0.08	0.78
Body mass (g)	0.40 ± 0.03	0.40 ± 0.02	0.02	0.80
Condition index	1.18 ± 0.03	1.16 ± 0.03	0.17	0.68
Total brain area (mm²)	8.55 ± 0.26	8.02 ± 0.24	2.20	0.15

Note: The df values are 1 and 38 for all comparisons. See text for details.

For northern redbelly dace, we conducted the same analysis as described above for Atlantic salmon. As our northern redbelly dace results included a wild-caught (WC) control group, we added two additional statistical comparisons. First, we included post hoc comparisons (least-squared difference) to the GLM tests of brain area. Second, we compared the Procrustes variance (morphological variation) for the shape data of the three groups (Adams 2018). The relative areas of the olfactory and optic regions, by treatment and corrected for standard length, were calculated as $\log_{10}$(area)/$\log_{10}$(length).

Results

Atlantic salmon

We found that Atlantic salmon from the HR and AR groups did not differ significantly from each other with respect to standard length, body mass, condition index, or total brain area (one-way ANOVA, $p > 0.05$; Table 1). The $\log_{10}$-transformed variables for regional areas, as well as the covariate, were normally distributed with respect to factors risk and enclosure (Shapiro–Wilk *W* test, $p > 0.05$). Multivariate GLM was insignificant for treatment (Pillai's trace = 0.13, $F_{[4,34]} = 1.24$, $p = 0.31$). Procrustes ANOVA found a significant effect of risk on brain shape ($F_{[1,17]} = 2.19$, $p = 0.02$), driven primarily by variation in the position of landmarks defining the optic tecta and cerebellum in the hind brain (Figs. 1A–1C), but not for overall centroid size or shape × size ($p > 0.8$ for both). The plotTangentSpace function reported 21 principal components (PC) with the proportion of variance for PC1 and PC2 being 0.34 and 0.15, respectively.

Northern redbelly dace

The HR, AR, and WC conditions were not significantly different in standard length, mass, or condition index (one-way ANOVA, $p > 0.05$; Table 2). There was a significant effect of treatment on total relative brain area (univariate GLM, $F_{[2,30]} = 8.94$, $p < 0.01$). As the olfactory bulbs were the region most frequently detached or damaged, we followed up with a GLM of total brain area without the olfactory bulbs vs. treatment, which was also significant ($F_{[2,68]} = 6.41$, $p < 0.01$). Post hoc least-squared difference testing showed that the HR fish had significantly larger brains than the AR (mean difference = 1.26 mm², $p < 0.01$) and WC (mean difference = 1.18 mm², $p = 0.01$) groups. As with the Atlantic salmon, the response variables were normally distributed (Shapiro–Wilk *W* test, $p > 0.05$).

Multivariate GLM analysis for all intact specimens was significant for risk (Pillai's trace = 0.559, $F_{[8,56]} = 2.713$, $p = 0.013$) and univariate GLM found a significant effect of treatment for the olfactory bulbs ($F_{[2,30]} = 5.62$, $p < 0.01$) and optic tecta ($F_{[2,30]} = 5.756$, $p < 0.01$) (Figs. 2A–2B). Follow-up univariate GLM of all specimens with intact optic tecta was also significant ($F_{[2,68]} = 8.248$, $p = 0.001$). The telencephalic palliums did not differ significantly in size across treatments, nor did the cerebella ($p > 0.05$).

Procrustes ANOVA found a marginally non-significant effect of treatment for both groups ($F_{[2,27]} = 1.69$, $p = 0.07$) and $\log_{10}$-transformed centroid size ($F_{[1,27]} = 1.7$, $p = 0.08$) (Figs. 3A–3C). The test of morphological disparity among the three groups showed the Procrustes variance (PV) for the AR group was significantly greater than for the HR and WC groups ($PV_{AR} = 0.004$, $PV_{HR} = 0.0025$, $PV_{WC} = 0.0025$, $p < 0.01$ for both).

Discussion

Our results suggest that exposure to conditions of elevated predation risk for as little as 2 weeks is sufficient to induce differential brain morphologies in two species of freshwater fish. Interestingly, our two focal species were at very different life-history phases (juvenile Atlantic salmon and sexually mature northern redbelly dace), consistent with the observation that teleosts retain neuroproliferation throughout their life. In Atlantic salmon, these differences were primarily detectable as a difference in shape, whereas in northern redbelly dace, differences in proportional investment in different brain regions were most pronounced.

In experiment 1, we employed hatchery-raised Atlantic salmon and exposure to heightened risk produced no significant differences in brain size relative to controls, suggesting similar degrees of investment in neural tissue; however, there was a demonstrable difference in brain shape. Brain regions, such as the optic tectum, are heterogeneous structures composed of various subregions and cell populations (Boulanger-Weill and Sumbre 2019). Just as differences in size reflect investment at the regional level, our observed differences in shape may reflect differential patterns of growth at the subregional level.

Our experiment with the northern redbelly dace, in some ways an inversion of our Atlantic salmon work, changed fewer abiotic parameters, with the primary alteration being the imposition of confinement to a single location within the pond. Here heightened risk produced larger brains compared with ambient risk and wild-caught controls with detectably larger olfactory and optic tecta, but did not result in a significantly distinct brain shape. However, the northern redbelly dace warp grids do show an expansion of the mid- and forebrain (to the left) or hindbrain (to the right) along the PC1 axis, which is seen as relatively larger squares on the grid. Although not detected at the level of significance by the Procrustes analysis, the relatively larger mean optic tecta and olfactory bulb sizes of the HR group are reflected by the preponderance HR morphologies found to left of the *y* axis on the PCA plot.

Although we cannot directly compare these two experiments due to the methodological differences that we have described, we can report that in both experiments, exposure to a sudden and prolonged exposure to conspecific skin extract resulted in distinct brain morphologies, which are in line with patterns of alteration seen in populations exposed to heightened predation pressure in previous studies. The northern redbelly dace exposed to heightened risk developed larger olfactory bulbs with results similar in magnitude to what was reported by Gonda et al. (2012) in freshwater ninespine sticklebacks exposed to simulated predation risk. These and our other results suggest that brain morphology is strongly influenced by environmental conditions, including predation (Ebbesson and Braithwaite 2012).

For our Atlantic salmon fry, the transition from the hatchery necessarily entailed a shift in living conditions, including a dramatic decrease in population density, a shift from artificial to natural lighting, and an altered water chemistry. Additionally, cessation of the supplemental feeding upon leaving the hatchery required the fry to feed on and potentially compete for drift fodder (Imre et al. 2005). Beyond the stresses of the transition, the HR fry may have been subject to non-consumptive effects of predation (NCEs), such as reduced foraging time and elevated stress (Elvidge et al. 2014; Elvidge and Brown 2015). We expected the HR fry to exhibit signs of these NCEs with potentially reduced standard length, mass, condition index, and (or) total brain size. That there was no effect of treatment for these outcomes may indicate that the duration or intensity of risk elevation was insufficient to produce noticeable negative effects (Archard et al. 2012; Elvidge et al. 2014).

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Table 2. Mean (±SE) values for parameters measured for northern redbelly dace (*Phoxinus eos*) in heightened risk, ambient risk, or wild-caught conditions.

	Ambient risk	Heightened risk	Wild-caught	*F*	*p*
Standard length (mm)	44.60 ± 0.47	43.60 ± 0.46	43.00 ± 0.07	2.07	0.13
Body mass (g)	1.20 ± 0.03	1.12 ± 0.03	1.08 ± 0.03	2.98	0.06
Condition index	1.35 ± 0.02	1.35 ± 0.02	1.36 ± 0.03	0.04	0.97
Total brain area (mm²)	12.74 ± 0.18	14.00 ± 0.26	12.82 ± 0.47	6.59	0.004

Note: The df values are 2 and 71 for all comparisons, except total brain area where df = 2 and 33. See text for details.

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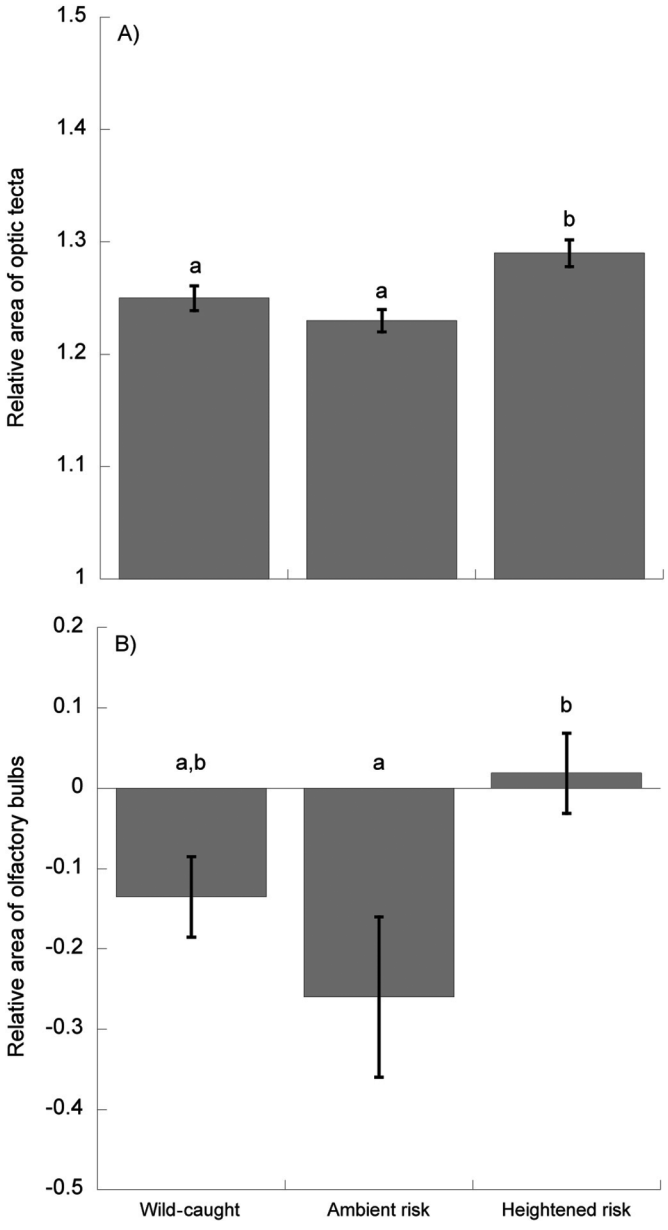
Fig. 2. The mean (±SE) relative regional areas of the (A) optic tecta and (B) olfactory bulbs by treatment for the northern redbelly dace (*Phoxinus eos*). Bars with the same letter do not significantly differ ($p > 0.05$).



In contrast with our Atlantic salmon work, the northern redbelly dace were wild-caught specimens and included the imposition of confinement; thus, preventing their natural diel feeding migrations and creating artificially maintained shoals (Naud and Magnan 1988). As their pond was static, we could not rely on sufficient drift fodder entering the northern redbelly dace enclosures and supplemental feeding was employed. Owing to this and the imposition of restricted movement, energy restriction was expected to be less critical than with the Atlantic salmon. Both treatment groups maintained their condition factors and separately exhibited traits indicative of growth and investment in somatic or neural tissue.

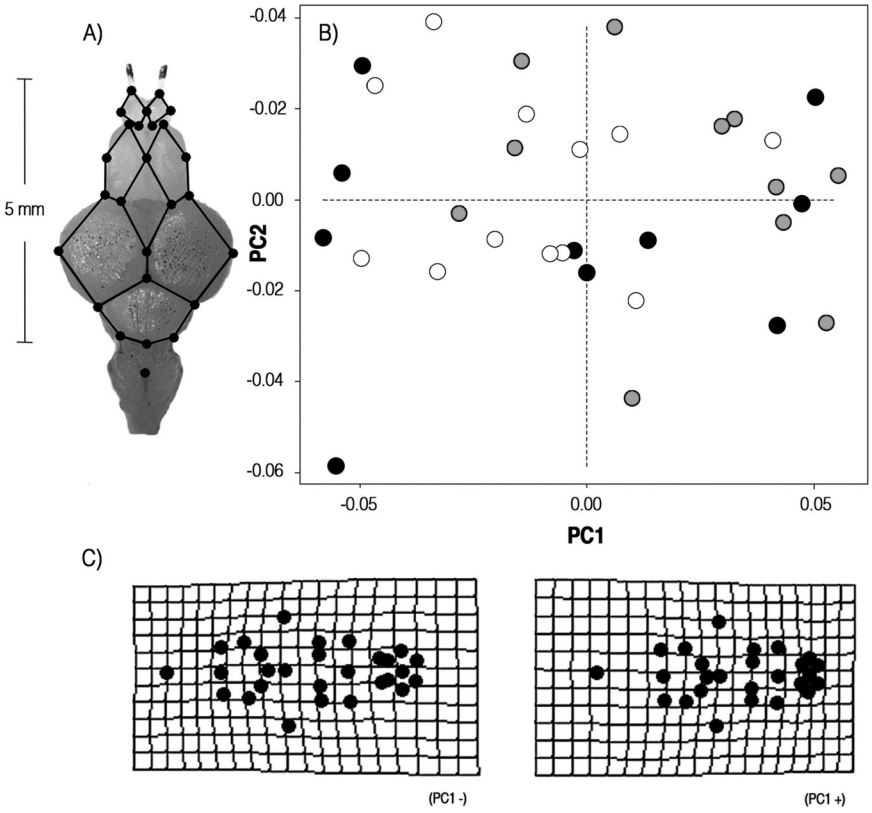
The AR group, confined and fed but not exposed to elevated risk, had the largest bodies and the most widely distributed brain morphologies. Within this group, brain shapes spanned the *x* axis of the PCA plot (Fig. 3B), resulting in a significantly greater Procrustes variance. Although effects of confinement (i.e., common garden and hatchery conditions) have been demonstrated in other species, we had no a priori expectations for our northern redbelly dace with regard to shape. As such, the holding conditions themselves may have influenced the final brain shape and further investigation is required to characterize their effects.

One potential consequence of confinement was the establishment of new social hierarchies within the bins. Changes in social status can alter patterns of gene expression in the brain in minutes (Maruska and Fernald 2010) Furthermore, social status has been shown to regulate growth rate in fish, with social ascent increasing growth rate and social descent slowing and potentially reversing it (Hofmann et al. 1999; Maruska and Fernald 2010; Elvidge et al. 2014). The wide variance of the AR group may reflect the effects of imposing an inescapable social group, raising the possibility of social rank as an additional variable in future studies.

We relied on the introduction of conspecific chemical alarm cues to simulate a stark increase in predation. The relative concentration of alarm cue used (~1.5 cm² of skin per exposure) roughly corresponds to the destruction of one individual conspecific in the immediate vicinity of its release. Relative to their previous predation risk (none for Atlantic salmon and presumed to be very little for northern redbelly dace), these concentrations of alarm cue likely represented the introduction of a new and voracious predator into their environment. However, owing to both of our model species having a high degree of olfactory sensitivity, coupled with the proximity of their neighbors and the natural settings of the experiments, our AR groups cannot be said to have been unexposed to predation. Studies with northern redbelly dace and Atlantic salmon suggest that both species are sensitive to degrees of perceived predation risk and exhibit responses proportional to its intensity (Dupuch et al. 2004; Blanchet et al. 2007; Wisenden 2008; Leduc et al. 2010). Our findings suggest that in both experiments the intensity of risk encountered by the HR groups was sufficiently elevated to result in differentiation.

Under these apparently life or death conditions, changes that reduce the odds of being eaten and their attendant NCEs become worthwhile (Lima and Bednekoff 1999; Benard 2004; Abrahams 2005). Inducible somatic defenses can take weeks or months to become fully effective and their appearance coincides with changes in other traits that can impose a cost for their implementation (Relyea and Auld 2004). For instance, the greater body depth of crucian carp (*Carassius carassius* (Linnaeus, 1758)) trades reduced swimming efficiency for reduced predation risk (Pettersson and Brönmark 1999; Vøllestad et al. 2004). The rapidity and flexibility of behavioural change in the face of predation is made possible

Fig. 3. Procrustes analysis of the northern redbelly dace (*Phoxinus eos*) showing (A) the configuration of landmarks and polygons used in the analysis overlaid on an example of a typical northern redbelly dace brain, which exhibits the sort of variation observed in (B) a principal components (PC) plot for brain shape by treatment (ambient risk (black circles), heightened risk (white circles), and wild-caught control (grey circles)) and (C) wireframe plots representing the shape configurations, at either end of the PC1 axis, as deformations (warps) of the mean shape for all specimens.



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by the brain. There is increasing evidence that this behavioural plasticity is underpinned by concurrent neuroplasticity. As a result, teleosts enjoy a remarkable capacity to cope with a changing world.

These experiments provide a point of reference for anticipating when differences may be detectable. Our results suggest that the teleost brain is more rapidly adaptable than has been previously reported. Further study is needed to determine to what extent these observed differences correlate with specific antipredator behaviour. Presently, our results may inform behavioural research more generally by highlighting how quickly environmental change produces macroscopic changes in morphology.

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