

Differences in brain morphology of brown trout across stream, lake, and hatchery environment

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Abstract

The trade-off between cognitive capacity and developmental costs drive brain size and morphology across fish species, but this pattern is less explored at intraspecific level. Physical habitat complexity has been proposed as a selection pressure on cognitive capacity that shapes brain morphology of fishes, but development of brain is also inherently linked to supply of energy and nutrients, particularly of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). In this study, we compared brain morphology of brown trout *Salmo trutta* from stream, lake, and hatchery environments, which differ in physical complexity and availability of dietary n-3 LC-PUFA ranging from low habitat complexity and high n-3 LC-PUFA availability in hatchery to high habitat complexity and low n-3 LC-PUFA availability in streams. We found that brain size, and size of optic tectum and telencephalon differed across the three habitats, being largest in lake fish. We suggest that these differences appeared to associate with diet quality and habitat specific behavioural adaptations rather than physical habitat complexity.

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Abstract

The trade-off between cognitive capacity and developmental costs drive brain size and morphology across fish species, but this pattern is less explored at intraspecific level. Physical habitat complexity has been proposed as a selection pressure on cognitive capacity that shapes brain morphology of fishes, but development of brain is also inherently linked to supply of energy and nutrients, particularly of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). In this study, we compared brain morphology of brown trout *Salmo trutta* from stream, lake, and hatchery environments, which differ in physical complexity and availability of dietary n-3 LC-PUFA ranging from low habitat complexity and high n-3 LC-PUFA availability in hatchery to high habitat complexity and low n-3 LC-PUFA availability in streams. We found that brain size, and size of optic tectum and telencephalon differed across the three habitats, being largest in lake fish. We suggest that these differences appeared to associate with diet quality and habitat specific behavioural adaptations rather than physical habitat complexity.

Key words

Omega-3 long chain fatty acids, animal cognition, intra-specific variability, trophic interactions, hatchery environment

Introduction

The brain is the anatomical structure that determines the information processing capacity and behavioral adaptation of animals (Kotrschal *et al.*, 1998; Pike *et al.*, 2018). Brain size is driven by the trade-off between benefits provided by cognitive skills and costs for its development and maintenance (Boogert *et al.*, 2018; Morand-Ferron *et al.*, 2015). Previous studies have shown that the fish brain often responds to selection pressure as a modular organ, in that only specific region controlling required cognitive skills under the selection will increase their volume, while the brain regions that are not used can reduce their volume as an energy saving adaptation (Kotrschal *et al.*, 2017; Pike *et al.*, 2018; Fong *et al.* 2021). Response of brain to physical habitat complexity has been proposed as one of the key drivers shaping brain morphology across different fish species, with more complex habitats selecting for larger brains and, particularly, for the larger brain regions that facilitate spatial navigation and complex decision making (*i.e.*, telencephalon), perception of visual cues (*i.e.*, optic tectum), and motor coordination (*i.e.*, cerebellum) (Kotrschal *et al.*, 1998; Pollen *et al.*, 2007). The changes in brain morphology of fishes can be evolutionary (Kotrschal *et al.*, 1998; Pollen *et al.*, 2007) as well as plastic (*e.g.*, Näslund *et al.*, 2012; Triki *et al.*, 2019). However, the association of physical habitat complexity and brain morphology in wild fishes has been much less studied at intraspecific than interspecific level.

A study on three-spined sticklebacks *Gasterosteus aculeatus* have shown differences in brain morphology among populations from lake and stream habitats (Ahmed *et al.*, 2017), but these differences were not always consistent with the prediction that individuals from more physically complex stream habitat have larger telencephalon than individuals from less complex lake habitat (Ahmed *et al.*, 2017). Lake fish can also experience high habitat complexity in lakes with developed littoral zone, but fish in lakes with simple shoreline should generally experience lower physical habitat complexity than stream dwelling conspecifics (Park & Bell 2010; Ahmed *et al.*, 2017). Therefore, what drives the differences in brain morphology among lake and stream dwelling populations of fishes, remains an open question. Development of brain morphology is inherently linked to supply of energy and nutrients, particularly of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) (Pilecky *et al.*, 2021). The availability of these nutrients differs across ecosystems, and they are more available to fish in lake habitats than in streams (Heissenberger *et al.*, 2010). High amount of dietary n-3 LC-PUFA is also typical for diet of hatchery reared fish (Heissenberger *et al.*, 2010), which are raised in habitats with extremely low physical complexity (Näslund *et al.*, 2012). Therefore, a comparison of brain morphology across individuals from stream, lake, and hatchery habitat can provide an insight into intraspecific responses of brain size and morphology to habitat quality in freshwater fishes.

Brown trout, *Salmo trutta* L., is a good model species for such comparative study, because genetically and phenotypically different populations of brown trout occur in lake and stream habitats (Jonsson & Jonsson 2011). A previous study has shown differences in brain morphology of anadromous and stream resident brown trout, which were suggested to be driven by differences in sex specific reproduction strategies rather than by physical habitat complexity (Kolm *et al.* 2009). Brown trout, like other salmonids, are also often reared in extremely simple hatchery environments (Heissenberger *et al.*, 2010; Näslund *et al.*, 2012). Some evidence suggests that hatchery-reared individuals have limited cognitive skills caused by a plastic response of their brain to the simplicity of the habitat in which they have developed (*e.g.*, Näslund *et al.*, 2012), and by an evolutionary response to the artificial selection pressure on the hatchery strains (*e.g.*, Fleming *et al.*, 2000). In this study, we aim to compare brain morphology of brown trout from stream, lake, and hatchery environments in order to test how brain morphology varies across environments that differ in physical habitat complexity and quality of available diet.

Materials and methods

Fish sampling and data collection

In January 2019 brown trout were captured in Scotland, UK, at Loch Sloy (56.2632175°N, 4.7707667°W; N = 14; FL = 261±52 mm mean±SD) and Carron Valley Reservoir (56.0338314°N, 4.1057406°W; N = 14; FL = 227±37 mm) using 30 m × 1.5 m single-mesh (38 mm) benthic gill nets. Three nets were deployed in late afternoon and retrieved the following morning. Loch Sloy has surface area of 1.33 km² and mean depth 25.5 m. Carron Valley Reservoir has surface area 3.76 km² and mean depth 9.6 m. Fish community in Loch Sloy is composed of brown trout, European whitefish *Coregonus lavaretus*, and European eel *Anguilla Anguilla*. Carron Valley Reservoir contains the same fish species as Loch Sloy complemented by rainbow trout *Oncorhynchus mykiss*, three-spined stickleback, and perch *Perca fluviatilis*. In October 2019 brown trout were captured by electrofishing (e-fish, UK) in small tributaries of Loch Lomond, Scotland, UK (56.0470272°N, 4.5504428°W): Ross Burn (N = 12, FL = 153±16 mm) and Wood Burn (N = 3, FL = 165±42 mm). Ross Burn has a mean discharge 0.3 m³.s⁻¹ and length 2.3 km and Wood Burn has a mean discharge 1.1 m³.s⁻¹ and length 3.1 km. Fish communities in Ross Burn and Wood Burn are the same, composed of brown trout, brook lamprey *Lampetra planeri*, European eel, and occasional Atlantic salmon *Salmo salar* and European minnow *Phoxinus phoxinus*. Finally, in January 2020 hatchery young-of-year brown trout (AE Fishery, Moffat, UK) were transported to the Scottish Centre for Ecology and the Natural Environment (SCENE). Hatchery trout were held in unadorned 120 L cylindrical tanks at a density of 20 fish per tank. Tanks were fed individually with water on a flow-through system directly from Loch Lomond at natural temperatures (low of ~3° C in winter to high of ~18° C in summer). Inflows of ~100 L per hour were angled to provide a current and an air stone was added to each tank. Lighting simulated ambient sunlight at the latitude of the facility (~56° N). Hatchery trout were fed daily to satiation on commercial salmon pellets containing 31.3 % of fish oil (Ewos Ltd., UK). These commercial pellets are a standard diet of hatchery trout and contain high amount of n-3 LC-PUFA (Heissenberger *et al.*, 2010). Hatchery fish were held under these conditions until June 2020, when brain samples of randomly selected individuals were extracted (N = 15, FL = 178±22 mm). All fish were killed with an overdose of benzocaine. Heads of fish were removed and fixed in 4% buffered (pH 6.9) paraformaldehyde solution. Brains were then dissected out by opening the skull along the anteroposterior axis and removing muscle tissue and bones around the brain until the brain could be lifted from the skull. Dissected brains were stored in 4 % buffered paraformaldehyde until further procedures were conducted. Brains were photographed with a Canon EOS 1300D DSLR camera with an EF-S18-55 III lens (Canon) and 13- and 31-mm extension tubes designed for Canon DSLRs (Xit Inc.). For each dissected brain sample, an image was taken from dorsal, left lateral and ventral views. Each brain was measured to calculate total volume and the volumes of the cerebellum, optic tectum, telencephalon, olfactory bulb, and hypothalamus. Measurements were completed using ImageJ 1.48 (Schneider *et al.* 2012) and used to calculate volume with the formulas outlined by Pollen *et al.* (2007).

We performed geometric morphometric analysis of body shape to confirm the predominant habitat use of wild caught individuals (see Appendix). This analysis indicated clear differences in body shape of individuals caught in lake and stream habitat (Procrustes ANOVA: $F_{2,64} = 11.6$, $p = 0.001$), which suggests that the presence of individuals in the sampled habitat (*i.e.*, lake or stream) was not coincidental, and it corresponds to their long-term habitat preference.

Statistical analysis

All analyses were conducted in R v.4.0.2 (R Core Team 2020). We tested the effect of habitat on overall brain volume using a linear model with habitat (categorical variable: lake, stream, hatchery), fork length, and their interaction. Effect of habitat on brain regions was tested by linear models with habitat and total brain volume minus the volume of the region of interest (Pike *et al.* 2018), and their interaction. Variables in all models were log-transformed. Non-significant interaction terms were removed from models. Significance of the explanatory variables was evaluated using ANOVA tables using Type II and III sums of squares for models without and with the interaction term, respectively. Model fit was diagnosed by control of distribution of model residuals and association of fitted and residual values. Differences between categories of habitat were analyzed using Tukey's HSD post-hoc test.

Results

Overall brain volume was affected by the interaction between habitat and the fork length (FL) of individuals ($F_{2,49} = 22.61$, $p < 0.001$, model $R_{adj.} = 0.947$). The significant interaction indicates that while there was a positive link between the overall brain volume and FL in wild populations *i.e.*, lake and stream habitat ($F_{1,37} = 214.28$, $p < 0.001$, model $R_{adj.} = 0.947$), hatchery fish showed no association between the brain volume and FL ($F_{1,13} = 0.2431$, $p = 0.6302$, model $R_{adj.} = 0.000$, Fig. 1a). Relative brain volume was larger in fish from lake than from stream habitat ($F_{1,37} = 5.4795$, $p = 0.0247$). The telencephalon volume increased with increasing volume of the whole brain ($F_{1,52} = 109.49$, $p < 0.001$, model $R_{adj.} = 0.912$, Fig. 1b); however; telencephalon volume also differed significantly between fish from different habitats ($F_{2,52} = 8.164$, $p = 0.001$). Specifically, the telencephala of stream dwelling trout were smaller than those of trout from both lake and hatchery environments (post-hoc $p < 0.006$), but the telencephala of lake and hatchery trout did not differ from each other (post-hoc $p = 0.252$). The volume of the optic tectum increased with the increasing volume of the whole brain ($F_{1,52} = 175.51$, $p < 0.001$, model $R_{adj.} = 0.931$, Fig. 1c), and optic tectum volume also differed significantly between fish from different habitats ($F_{2,52} = 3.723$, $p = 0.031$). The optic tecta of lake dwelling trout were significantly larger than those of stream trout (post-hoc $p = 0.027$) and tended to be larger than the optic tecta of hatchery trout (post-hoc $p = 0.054$); but stream and hatchery trout did not differ from each other (post-hoc $p = 0.733$). Similarly, volumes of the olfactory bulb, cerebellum and hypothalamus increased with the increasing volume of the whole brain (olfactory bulb: $F_{1,52} = 61.42$, $p < 0.001$, model $R_{adj.} = 0.808$, Fig. 1d; cerebellum: $F_{1,52} = 80.70$, $p < 0.001$, model $R_{adj.} = 0.847$, Fig. 1e; hypothalamus: $F_{1,52} = 51.65$, $p < 0.001$, model $R_{adj.} = 0.651$, Fig. 1f), but volumes of these brain regions did not differ among individuals from different habitats (olfactory bulb: $F_{2,52} = 0.639$, $p = 0.532$; cerebellum: $F_{2,52} = 1.421$, $p = 0.251$; hypothalamus: $F_{2,52} = 2.403$, $p = 0.100$).

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Figure 1 – *The relationship between a) overall brain volume and fork length (i.e., encephalization), and between overall brain volume and volume of b) the telencephalon, c) optic tectum, d) olfactory bulb, e) cerebellum, f) hypothalamus.*

Discussion

The relative brain volume, *i.e.* after controlling for fish FL, was larger in lake habitats compared to streams. In addition, volumes of telencephalon and optic tectum were, in relation to other brain regions, disproportionately larger in lakes compared to streams, while volumes of other brain regions changed in close correlation to the overall brain volume. The telencephalon of stream fish was even smaller than telencephalon of hatchery individuals. Similarly to the study of Ahmed et al. (2017) on wild populations of stickleback, our findings do not support the hypothesis that in brown trout volume of brain and regions important for navigation and decision-making increases in physically complex stream compared to simpler lake and hatchery habitat.

We posit that the discrepancy between theoretical predictions and our findings can be explained by two main mutually non-exclusive factors. First is the non-linear association between physical habitat complexity and selection for larger brains, particularly for larger telencephala and optic tecta (Boogert *et al.* , 2018). This explanation assumes that habitat complexity beyond a certain threshold may favor simple behavioural strategies to operate effectively in those complex environments, because their success probability is comparable to more complicated and cognitively demanding behaviours requiring costly investment in brain development (Morand-Ferron et al. 2015). Therefore, the high complexity of the rapidly changing stream habitat may favour simpler behavioural strategies than the less complex lake habitat. Stationary behaviour and a sit-and-wait foraging strategy of stream dwelling trout (Jonsson & Jonsson 2011) is an example of such simple behavioural adaptations to the complex stream habitat. In contrast, alternation in foraging between pelagic and littoral zone and more common piscivory of lake-dwelling brown trout (Sánchez-Hernández 2020) may induce selection for large telencephala and optic tecta due to the need for visual cues processing and relatively complex navigation and decision-making skills that they require (Edmunds *et al.* , 2016).

The second possible explanation for differences in brain size between the habitats is differences in available diet quality, which limit the nutrient supply for brain development. Lake-dwelling trout feeding on zooplankton or other fish acquire more n-3 LC-PUFA than stream-dwelling trout, which rely on a mix of macro-zoobenthos and n-3 LC-PUFA-poor terrestrial insect (Heissenberger *et al.*, 2010; Sánchez-Hernández & Cobo 2016). Previous laboratory studies that have shown that availability of dietary n-3 LC-PUFA has positive effect on overall brain size (Lund *et al.*, 2012) and on size of optic tecta (Ishizaki *et al.*, 2001). Therefore, the higher dietary intake of these nutrients may facilitate brain size development in lake-dwelling brown trout, compared to their conspecifics from stream habitats. An extremely n-3 LC-PUFA-rich diet is also typical for hatchery-reared fish (Heissenberger *et al.*, 2010). This high-quality diet may loosen the selection trade-off between the cost of brain development and benefits of high cognitive capacity that typically shapes brain morphology in wild animals (Morand-Ferron *et al.* 2015; Boogert *et al.*, 2018). Thus, a high-quality diet, which enables rapid brain growth in hatchery fish, can explain the findings of this and other studies (Näslund *et al.*, 2012; Kotrschal *et al.*, 2012) that hatchery salmonids can have similar or larger brain and telencephalon volume than stream-dwelling salmonids, despite the low physical complexity of hatchery habitat. The relative brain size (*i.e.*, encephalization) of hatchery trout was in our study difficult to compare with the wild fish due to the difference of allometric relationship between the FL and brain in hatchery and wild individuals, but it appears that hatchery fish brain was of intermediate size between fish of stream and lake origin. The lack of positive correlation between the brain size and FL in hatchery individuals is unusual for wild fishes where body size is a strong predictor of brain volume (*e.g.*, Triki *et al.* 2021). Previous studies on evolution of encephalization in mammals have suggested that relative brain size depends on selection pressure on size of the brain as well as on the overall body size (Smaers *et al.* 2012). Therefore, high-quality diet and selection for fast body growth in hatchery fish could uncouple the developmental link between the brain and the rest of the body (Kotrschal *et al.*, 2012; Smaers *et al.* 2012).

Other factors that have been shown to influence brain size and morphology in fishes and were not explicitly considered in our comparative study are predation pressure (Kotrschal *et al.*, 2017), sex (Kolm *et al.*, 2009; Näslund 2018), and ontogeny (Abrahao *et al.* 2021). Potential predators, that is eel and large trout, were present in all sampled wild populations (see method section), and thus were unlikely to explain differences between the lake and stream habitat. Proportion of males and females in brown trout populations is generally even (*e.g.*, Baglinière *et al.* 1989) and thus should not differ between the groups compared in this study, but some studies suggest that female brown trout are more common in pelagic lake habitat (*e.g.*, Jonsson 1989). There were clearly ontogenetic differences between the compared groups, as hatchery trout were young-of-year, while the sizes of all wild trout corresponded to adults. In addition, lake trout were larger than stream trout, and thus some of lake individuals could have been older than the rest of the sample, but these differences could also stem from differences in growth rates in lake and stream trout (Jonsson & Jonsson 2011; Sánchez-Hernández 2020). The differences in growth rates among the environments compared in this study makes it impossible to collect individuals of the same body size and age, and thus confounding of these two factors is an inherent shortcoming of any such comparative study.

In conclusion, our study provides an example of among population variability of brain size and morphology, which is a topic still widely understudied in the wild. We suggest that besides the cognitive demands of the environment (*e.g.*, habitat complexity) future studies should also consider the availability of dietary essential fatty acids as a possible key driver of brain evolution and development in wild fishes.

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Competing interests

Authors have no conflict of interests to declare.

Contribution

LZ conceived, collected samples and performed statistical analyses, and drafted the manuscript; JPK conceived and coordinated the study, collected samples and performed morphometric analysis; TAA and LF performed brain extraction and photography; CEA conceived the study and collected samples. All authors critically revised the manuscript and gave final approval for publication and agree to be held accountable for the work performed therein.

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Data availability

Should the manuscript be accepted data will be archived at figshare.com (doi: xxxxxxxxxxxx).

Appendix

Table S1. Pairwise comparisons of LS-means distances derived from Procrustes ANOVA (RRPP: 1,000 permutations) describing shape differentiation between brown trout from three habitat types

Pairwise comparison	Distance	UCL (95%)	Z	p
Lake – Stream	0.0392	0.0560	9.787	0.001
Lake – Hatchery	0.0331	0.0138	9.367	0.001
Stream – Hatchery	0.0256	0.0142	6.388	0.001

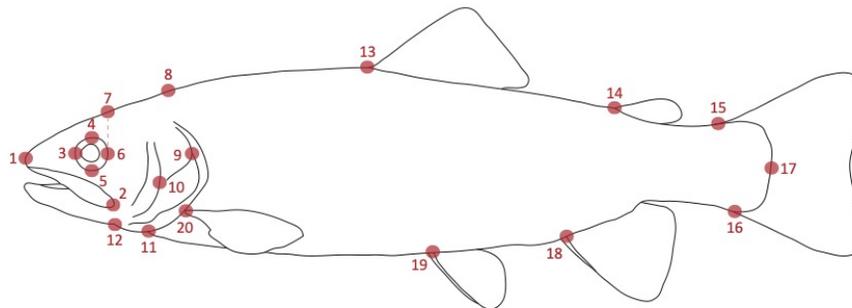


Figure S1. Landmark placements for geometric morphometric analyses: (1) tip of snout; (2) posterior tip of maxilla; (3–6) anterior, superior, inferior and posterior of eye; (7) superior of cranium perpendicular to 6, indicated by broken line; (8) superior posterior of cranium; (9) anterior intersection of opercle and subopercle; (10) inferior intersection of opercle and preopercle; (11) ventral margin of opercula; (12) ventral margin of mandible; (13) anterior insertion of dorsal fin; (14) anterior insertion of adipose fin; (15–16) superior and inferior insertions of caudal fin; (17) junction of caudal fin at lateral line; (18) anterior insertion of anal fin; (19) anterior insertion of pelvic fin; (20) anterior insertion of pectoral fin.

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image3.emf available at <https://authorea.com/users/428827/articles/532622-differences-in-brain-morphology-of-brown-trout-across-stream-lake-and-hatchery-environment>

Figure S2. PCA plot of brown trout body shape, corrected for allometry (regressing Procrustes coordinates on centroid size) and lunate bending in PC2 (regressing coordinates on PC2 scores), and coloured by habitat type.

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Figure S3. Average body shapes of brown trout by habitat type (all exaggerated 2x for clarity).