

Environment-dependent plasticity and ontogenetic changes in the brain of hatchery-reared Atlantic salmon

J. Näslund¹, M. H. Larsen^{2,3}, S. T. Thomassen³, K. Aarestrup² & J. I. Johnsson¹

¹ Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

² National Institute of Aquatic Resources, Section for Freshwater Fisheries and Ecology, Technical University of Denmark, Silkeborg, Denmark

³ Danish Centre for Wild Salmon, Randers, Denmark

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Correspondence

Joacim Näslund, Department of Biological and Environmental Sciences, University of Gothenburg, PO Box 463, SE-405 30 Gothenburg, Sweden.
Email: joacim.naslund@gmail.com

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Introduction

Stocking of hatchery-reared salmonids to enhance or re-establish populations has been a common practise in fisheries, conservation and restoration programmes for decades, but the relevance of these programmes has been questioned due to the low survivability of stocked fish as compared to wild conspecifics (Schuck, 1948; Jonsson & Jonsson, 2011). Particular critique has been directed to the fact that many stocking programmes are more concerned with the number of fish to be stocked rather than their quality and the number of surviving fish (e.g. Johnsson, Brockmark & Näslund, 2014). The survival rates of stocked individuals are commonly less than half of those of wild fish with the same genetic origin (Jonsson & Jonsson, 2011). Particularly high mortality rates of hatchery salmonids are seen soon after the release (Saloniemi *et al.*, 2004; Aarestrup *et al.*, 2014; Melnychuk *et al.*, 2014). Consequently, several recent studies have focused on rearing methods aiming to modify the hatchery environment for the production of wild-like fish with higher post-stocking survivability (Brännäs & Johnsson, 2008; Johnsson *et al.*, 2014). Fish density reduction in the rearing tanks is a promising modification, which repeatedly has been shown to increase the post-release survival of salmonids (Brockmark & Johnsson, 2010; Brockmark, Adriaenssens & Johnsson, 2010; Barnes *et al.*, 2013; Kavanagh & Olson, 2014; Larsen *et al.*, 2016; but also see Clarke, Cameron & Carmichael, 2013). Improved survival of fish reared at reduced densities is, at least partially, believed to be a consequence of development of higher cognitive capacity as well as expression

Abstract

Lowered rearing density has repeatedly been shown to increase the performance of hatchery-reared salmonids stocked into natural environments. One possible mechanism for this pattern could be that lower densities enhance brain development, which has been shown to be the case in other hatchery enhancement strategies, like environmental enrichment. Here, we investigated the size of the brain in hatchery-reared Atlantic salmon *Salmo salar* kept at standard (high) and reduced (low) tank densities. In contrast to our predictions, we found that fish reared at high density had larger dry mass of cerebellum and telencephalon, correcting for body size. No differences were detected for total brain mass. Furthermore, we found that the relative size of both telencephalon and cerebellum, in relation to total brain mass, changed with body size. Cerebellum increased in relative size with increased body size, while the opposite pattern was observed for telencephalon. Overall, these results reveal substantial brain plasticity depending on the surrounding environment as well as ontogenetic adaptive changes in the brain of the Atlantic salmon.

of behaviours more suited for the natural environment (Brockmark *et al.*, 2010; Johnsson *et al.*, 2014). Lower tank densities may, for instance, provide a social environment where interactions among individuals are more similar to the natural environment, allowing for individual recognition and social learning (Griffiths *et al.*, 2004; Chapman, Ward & Krause, 2008; Brockmark & Johnsson, 2010). Lowered densities may also reduce crowding stress and related physiological problems (Ellis *et al.*, 2002; Ashley, 2007; Rosengren *et al.*, 2016).

In hatchery-reared salmonids, studies of brain size have suggested positive effects of environmental enrichment in captive environments (Näslund & Johnsson, 2016), potentially driven by increased cognitive stimulation and brain cell proliferation in more complex environments (Salvanes *et al.*, 2013). Intra-specific brain size variation in teleost fishes can depend on both genetic inheritance (guppy *Poecilia reticulata*: Kotrschal *et al.*, 2013; Chen *et al.*, 2015) and the environment (Atlantic salmon *Salmo salar*: Salvanes *et al.*, 2013; shortfin molly *Poecilia mexicana*: Eifert *et al.*, 2014), and is possibly associated with cognitive capacity (Kotrschal *et al.*, 2013; but see Healy & Rowe, 2013). Furthermore, studies in three-spined sticklebacks *Gasterosteus aculeatus* show that there can be substantial independence in growth among different brain regions, allowing for independent responses to environmental factors through phenotypic plasticity (Noreikiene *et al.*, 2015).

Here, we investigated brain size of hatchery-reared Atlantic salmon sampled from an experiment aimed at investigating post-release survival of fish reared at normal or reduced density. In this

experiment, reduced density led to increased survival during seaward migration, when the fish were released into a natural stream just prior to the onset of the physiologically induced migration (see Larsen *et al.*, 2016). We specifically investigated the size of telencephalon and cerebellum as these substructures previously have been shown to be affected by the hatchery rearing environment in salmonid fish (Lema *et al.*, 2005; Kihlslinger, Lema & Nevitt, 2006; Kihlslinger & Nevitt, 2006). The relative sizes of brain substructures are generally associated with the relative importance of the associated functions of the substructures in fish (e.g. Kotschal, van Staaden & Huber, 1998; Ito *et al.*, 2007). However, the specific functions of distinct brain substructures are convoluted; in general, specific substructures have multiple functions (Striedter, 2005). Nevertheless, based on multiple lines of evidence, the telencephalon is commonly assumed to govern several cognitive functions (Broglia, Rodriguez & Salas, 2003; Rodríguez *et al.*, 2006; Ebbesson & Braithwaite, 2012) and the cerebellum is involved in motor coordination (Kotschal *et al.*, 1998; Roberts, Dean & Paul, 2002) as well as some cognitive functions (Rodríguez *et al.*, 2006). We also investigated whole-brain size, as some studies suggest effects of rearing environment on the overall size (Näslund *et al.*, 2012; Brown *et al.*, 2013). We hypothesised that brain growth, in particular telencephalic and/or cerebellar growth, would be affected by social interactions. Based on results from previous studies, lower density was assumed to convey more social stimulation as a consequence of higher potential for individual recognition, resource defence and individual decision making in a less crowded environment (Brockmark *et al.*, 2010; Johnsson *et al.*, 2014). Consequently, we predicted that low-density fish would have larger brains, or larger brain substructures related to cognitive ability previously shown to be positively affected by increased environmental complexity (cerebellum: Kihlslinger & Nevitt, 2006; telencephalon: Kihlslinger *et al.*, 2006; Salvanes *et al.*, 2013).

The fish in our study showed bimodal size distribution, which is common in hatchery environments (e.g. Thorpe, 1977; Jonsson & Jonsson, 2011). At the time of sampling, the fish in the upper modal group, but not in the lower modal group, were generally going through smoltification (the physiological transformation for a life in marine environment) (Larsen *et al.*, 2016). Previous studies have indicated that the brain undergoes major changes during smoltification (Ebbesson & Braithwaite, 2012). To investigate whether ontogenetic changes in gross brain morphology also occur at this stage, we explored effects of body size on the size of telencephalon and cerebellum in relation to total brain size.

Materials and methods

Rearing and handling of the fish is described in detail in Larsen *et al.* (2016). In short, the fish were F1 offspring from 47 females and 27 males, caught in River Storå, Denmark, in autumn 2011. From the fry stage, two density treatments were used – high density (HD: 7500 fish m⁻²) and low density (LD: 2500 fish m⁻²) – each replicated three times in conventional indoor hatchery tanks (2 × 2 m, water level 35 cm) (Danish Centre for Wild Salmon, Randers, Denmark). On 16 June 2012, the density of fish was reduced to 1500 fish m⁻²

and 500 fish m⁻² in HD and LD tanks respectively. Water temperature (recirculating system, 30 L min⁻¹) and light regime followed natural cycles.

On 20 March 2013, 12 fish were sampled randomly from each tank (i.e. 36 fish per treatment), euthanized by a benzocaine overdose and decapitated after recording total body length (from the tip of the nose to the end of the spread-out caudal fin; precision: 1 mm) and body mass (precision: 0.1 g). Heads were preserved in 4% phosphate-buffered formaldehyde and stored at 4°C. To facilitate standardized separation of cerebellum from the rest of the brain (as judged from previous pilot dissections of salmonid brains), the heads were dorsoventrally bisected along the midsagittal plane using a scalpel on 25 June 2014 (Fig. 1a).

On 20 July 2015, the brains were dissected out of each half of every head. The cerebellum and telencephalon were separated from the rest of the brain (see Fig. 1). Cerebellum, telencephalon and the rest of the brain (see Fig. 1c) were all separately dried (right and left parts added together) in aluminium foil at 70°C for 35 h and thereafter the dry mass was recorded to the nearest 0.01 mg (Precisa XR 205SM-DR; Precisa Gravimetrics AG, Dietikon, Switzerland).

Statistical analyses

Whole-brain dry mass

The dry mass of the whole brain (i.e. telencephalon, cerebellum and the rest of the brain added together; $n_{HD} = 35$, $n_{LD} = 36$) was analysed using a general linear model with body length (L) as a covariate and density treatment (D) as a two-level fixed factor. The whole-brain dry mass appeared to have a linear relationship with body length (see Fig. 2) and was consequently not log-transformed. Tank effects were initially tested using tank (T) as a fixed factor nested within D [$T(D)$]. As no tendency for effect was detected ($P > 0.9$), it was subsequently removed from the analysis. The interaction between D and L ($T \times D$) was also initially included, but not retained in the final model ($P > 0.2$). Residuals from the model were found to be slightly negatively skewed; however, both density groups showed the same distribution pattern and the analysis was therefore retained.

Telencephalon dry mass

The dry mass of telencephalon ($n_{HD} = 36$, $n_{LD} = 36$) was analysed the same way as the whole brain. No tank effects were detected ($P > 0.8$) and neither were there any evidence of a $T \times D$ interaction ($P = 0.24$). For this reason, these terms were excluded in the final model.

Cerebellum dry mass

The dry mass of cerebellum ($n_{HD} = 35$, $n_{LD} = 36$) was analysed the same way as the whole brain. No tank effects were detected ($P > 0.9$) and neither were there any evidence of a $T \times D$ interaction ($P = 0.12$). These terms were therefore not included in the final model.

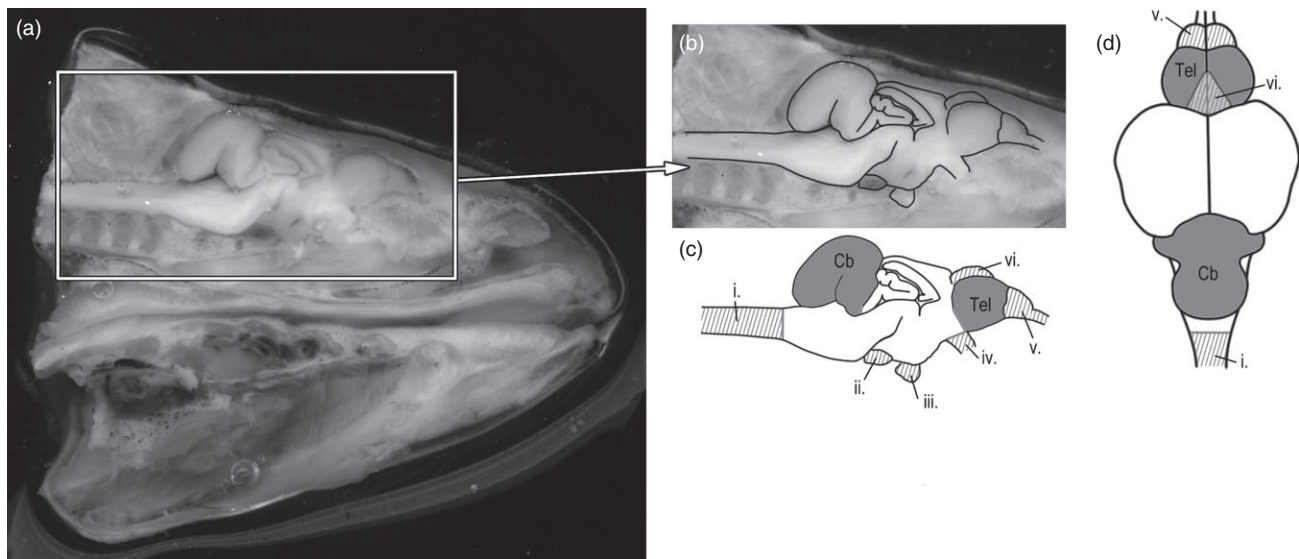


Figure 1 Illustration of the brain dissection scheme. (a) Midsagittal cut through the head of an Atlantic salmon. (b) Outlined main brain substructures. (c) Midsagittal and (d) dorsal schematic illustrations of the brain substructures included (Cb, Tel and 'rest of the brain') and excluded (i–vi) in the analysis. In grey: Cb – cerebellum (corpus cerebelli, crista cerebellaris and eminentia granularis, not including valvula cerebellaris); Tel – telencephalon. In white: 'rest of the brain' (i.e. the parts of diencephalon, mesencephalon and rhombencephalon not specifically mentioned; see fig. 15.4a–d in Meek & Nieuwenhuys, 1998). Striped (excluded): i, medulla spinalis; ii, saccus vasculosus; iii, hypophysis (pituitary gland); iv, nervus opticus; v, bulbus olfactorius and nervus olfactorius; vi, saccus dorsalis and epiphysis (pineal gland). Terminology adapted from Meek & Nieuwenhuys, 1998.

Size of telencephalon and cerebellum in relation to whole-brain size

To compare the proportional size of telencephalon and cerebellum in relation to the whole brain, we divided the dry mass of each substructure with the dry mass of the whole brain. The resulting quotients were analysed using the same model and term-selection procedure as in the analyses above. No tank effects were detected ($P > 0.35$) and neither were there any evidence of a $T \times D$ interaction ($P > 0.57$), and consequently these terms were not included.

General notes

During the dissection and weighing, the experimenter (JN) was blinded with respect to treatment.

All statistical analyses were carried out in IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA). Handling, rearing, tagging and sampling of fish were conducted in accordance to the guidelines described in permission (2012-DY-2934-00007) from the Danish Experimental Animal Committee.

Results

Whole-brain dry mass

Whole-brain dry mass was strongly positively related to body length ($F_{1,68} = 1000$, $P < 0.001$), but no effects of density treatment on whole-brain dry mass were detected ($F_{1,68} = 0.026$, $P = 0.87$) (Fig. 2a,b).

Telencephalon dry mass

Telencephalon dry mass was positively related to body length ($F_{1,69} = 470$, $P < 0.001$) and found to be higher in the HD treatment ($F_{1,69} = 5.3$, $P = 0.024$) (Fig. 2c,d).

Cerebellum dry mass

Cerebellum dry mass was positively related to body length ($F_{1,68} = 840$, $P < 0.001$) and found to be higher in the HD treatment ($F_{1,68} = 4.7$, $P = 0.033$) (Fig. 2e,f).

Size of telencephalon and cerebellum in relation to whole-brain size

The proportional size of both telencephalon and cerebellum changed with size of the fish ($F_{1,68} > 45$, $P > 0.001$). The proportional size of telencephalon decreased with size (Fig. 3a) and cerebellum increased with size (Fig. 3b).

In concordance with the previous analyses where body size was corrected for, the HD treatment had relatively larger size of both telencephalon ($F_{1,68} = 5.9$, $P = 0.018$; Fig. 3a) and cerebellum ($F_{1,68} = 10$, $P = 0.002$; Fig. 3b) when correcting for overall brain size.

Discussion

The results presented here demonstrate that Atlantic salmon juveniles reared at the higher density had larger cerebella and telencephala as compared to fish reared at the lower density,

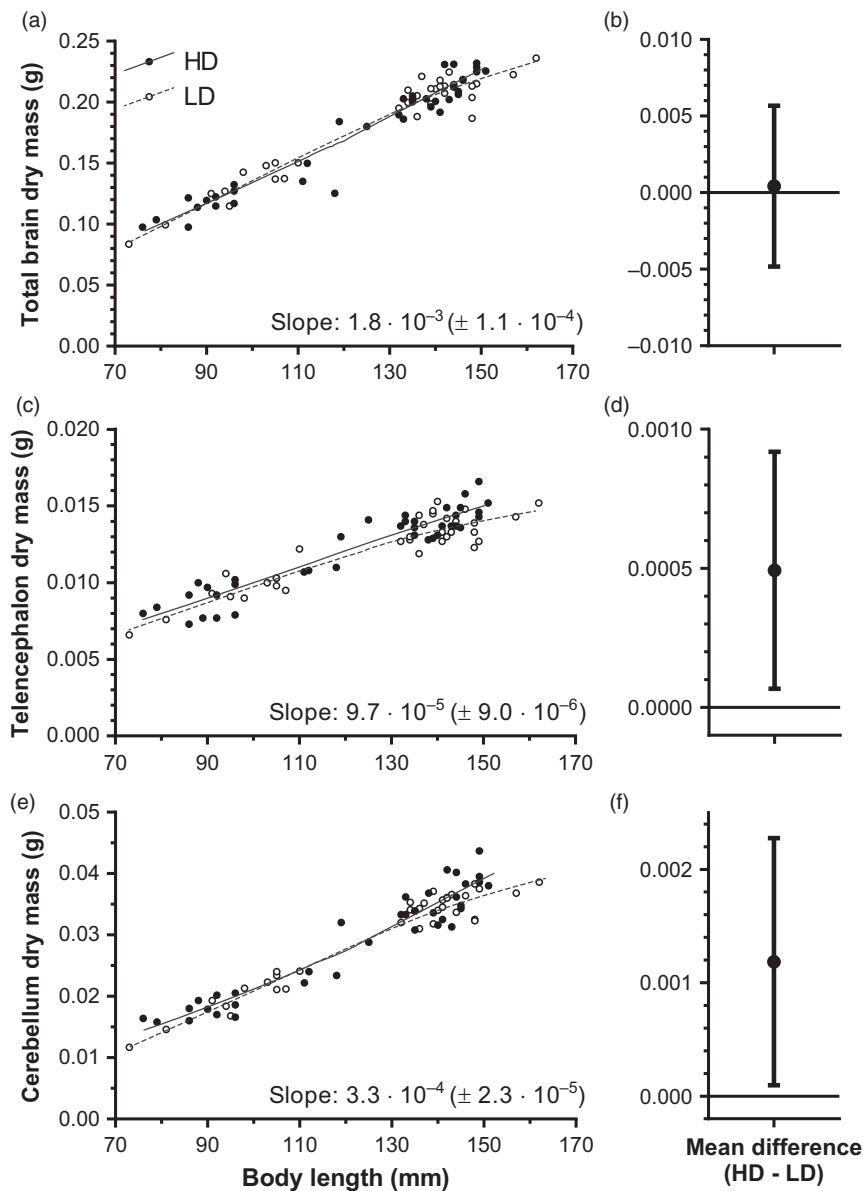


Figure 2 Relationship between body length and (a) total brain, (c) telencephalon and (e) cerebellum dry mass $\times 10$. Plotted lines represent local regressions (LOESS; Epanechnikov kernel, 90% of points to fit); the general slopes ($\pm 95\%$ confidence interval) are parameter estimates for the covariate L (body length) in the linear models (linear regression lines not shown). Mean differences ($\pm 95\%$ confidence interval) based on estimated marginal means from linear models are presented in (b), (d) and (f) respectively (0 = no difference). HD = high density; LD = low density.

both relative to body size and relative to overall brain size. No differences were detected in total brain size between the density treatments. Furthermore, we demonstrate a change in relative mass of cerebellum and telencephalon, compared to total brain mass, with body size. The relative mass of the cerebellum increased with body size, while the relative mass of the telencephalon decreased with size. These latter changes may be related to the smoltification process (Ebbesson & Braithwaite, 2012).

Rearing environment affects the brain

Studies on guppies suggest a link between overall brain size and cognitive capacity (Kotrschal *et al.*, 2013). Judging from other studies, both telencephalon and cerebellum are specifically associated with cognition in fish (Salas *et al.*, 1996; Rodríguez *et al.*, 2006; Ebbesson & Braithwaite, 2012). Regarding environmental effects on cognitive ability, studies on brown trout *Salmo trutta* have shown that lowered tank

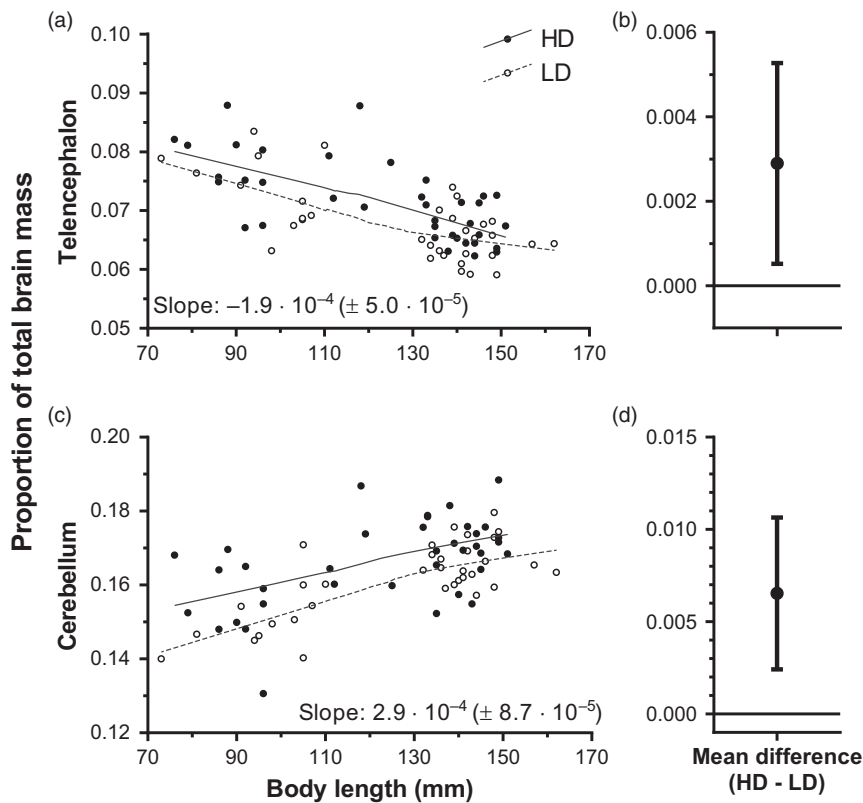


Figure 3 Relationship between body length and (a) proportional dry mass of telencephalon and (c) cerebellum in relation to total brain dry mass. Plotted lines represent local regressions (LOESS; Epanechnikov kernel, 90% of points to fit); the general slopes ($\pm 95\%$ confidence interval) are parameter estimates for the covariate L (body length) in the linear models (linear regression lines not shown). Mean differences ($\pm 95\%$ confidence interval) based on estimated marginal means from linear models are presented in (b) and (d) respectively (0 = no difference).

density could increase cognitive ability (Brockmark *et al.*, 2010) and thus we hypothesised that growth of telencephalon and cerebellum could be stimulated at lower density. However, the results did not support this hypothesis. Instead, we provide contrasting evidence that the average mass of telencephalon and cerebellum of the high-density fish were higher than for low-density fish. We specifically note that the higher migration success of individuals reared in the low-density treatment, demonstrated in Larsen *et al.* (2016), was neither matched by larger brains nor with larger telencephala or cerebella, which indicates that brain size may not be a suitable indicator of post-release performance.

Interestingly, stocking density in aquaria influence brain size of juvenile daffodil cichlids *Neolamprologus pulcher* with fish in denser groups developing larger cerebellum and hypothalamus, but smaller tectum opticum and no significant effects on telencephalon, dorsal medulla or bulbus olfactorius (Fischer *et al.*, 2015). Thus, cerebellum growth may be stimulated in higher density environments, perhaps related to increased demands on locomotor performance and manoeuvrability in relation to other individuals (Roberts *et al.*, 2002). However, while the results from Fischer *et al.* (2015) are partly consistent with our study and thus could suggest some generality of the effects of fish density, direct comparisons could be

inappropriate; first, due to group sizes being much lower in the cichlid study, and second, due to the differences in evolutionary history and ecology between the species.

We found no differences in total brain mass, which suggests that there is no general differential energy allocation to brain growth between the density groups, assuming that all brain regions are equally expensive to build. The larger size of cerebellum and telencephalon in the high-density treatments along with the similarity in total brain dry mass between treatments opens for the possibility that other brain substructures than the ones specifically investigated here are relatively larger in low-density fish. Potentially, there may be trade-offs in growth among different brain structures, which may give rise to the effects detected here. Additional studies are needed to investigate this.

Studies comparing hatchery-reared fish with wild, or semi-naturally reared, fish have provided mixed evidence for the direction of the effects of environment on brain size. In Atlantic cod *Gadus morhua*, hatchery-reared individuals with wild parentage had lower relative brain mass than wild conspecifics (Mayer *et al.*, 2011). Similar effects have been seen in the salmonid family, of which the Atlantic salmon is a member. For instance, domesticated rainbow trout *Oncorhynchus mykiss* have been shown to have smaller sizes of several brain

structures than wild conspecifics (Marchetti & Nevitt, 2003; Brown *et al.*, 2013). Judging from these studies, the general wild-type brain is larger than the hatchery-type brain, implying that hatchery fish potentially could be maladapted to a natural environment. However, several recent studies have, in contrast, shown that hatchery-reared fish end up with larger relative brain size than conspecifics reared in natural, or semi-natural, environments. For instance, Atlantic salmon individuals being reared continuously in the hatchery (the same hatchery as in the present study) had a higher brain:body mass ratio than individuals that were released into the wild half a year earlier (Näslund *et al.*, 2012). Hatchery-reared chinook salmon *Oncorhynchus tshawytscha*, originating from wild parents and being released as pre-smolts, were found to have larger relative brain size than wild conspecifics, even after 3 years at sea (Wiper, Britton & Higgs, 2014). Investigating male clonal lines of rainbow trout, domesticated lines (>10 generations in hatchery) had larger brains than wild lines (wild parents) (Campbell *et al.*, 2015). Furthermore, coho salmon *Oncorhynchus kisutch* reared in a semi-natural stream channel were found to develop smaller brains than hatchery-reared fish (Kotrschal *et al.*, 2012). In the same study, growth hormone transgenic coho were shown to develop smaller cerebella in the semi-natural channel than in hatchery tanks, which is consistent with the present study since the fish density in the hatchery tanks was lower. Altogether, the evidence for larger brains in wild or semi-naturally reared fish (with respect to both environmental complexity and fish density) is equivocal and requires more investigations.

Ontogenetic effects on the relative size of telencephalon and cerebellum

We found that the relative size of cerebellum, in relation to the total brain size, increased with body size. This effect could be related to the smoltification, when salmonid fish transform morphology and physiology for a life in marine environments (McCormick, 2013). Heightened levels of neural plasticity and reorganization in the brain have also been repeatedly shown in smoltifying salmonids, albeit mainly in the telencephalon (reviewed in Ebbesson & Braithwaite, 2012). The size distribution of the experimental population was bimodal, and the upper size mode contained the fish undergoing smoltification, while the lower mode likely contained mainly fish delaying their smoltification to the next year (Thorpe, 1977; Metcalfe, 1998; authors' observations). A previous study on wild brown trout show that anadromous adult individuals have relatively larger cerebella than stream-resident adult individuals (Kolm *et al.*, 2009), which suggests an adaptation to a more mobile life in the marine environment where good manoeuvrability is of high importance to catch prey and avoid predation.

In contrast to the cerebellum, the telencephalon decreased in size relative to the whole brain with increased body size. This effect was not detected in comparisons between anadromous and stream-resident brown trout (Kolm *et al.*, 2009). The apparent difference between these species may, however, be a consequence of their different habitat choice in the sea. Brown

trout post-smolts live in coastal areas of the sea, where environmental complexity is a feature of the environment, while Atlantic salmon post-smolts live in a pelagic environment devoid of structural complexity (Jonsson & Jonsson, 2011). Thus, a reduced size of telencephalon in Atlantic salmon smolts may be a consequence of reduced need for cognitive capacity in a pelagic marine environment being less complex than stream or coastal environments (see e.g. Shumway, 2008).

Conclusions

We conclude that higher stocking density in hatchery tanks results in larger sizes of telencephalon and cerebellum in the salmon brain. As a consequence of this result, we put forward the new hypothesis that growth of these structures may be stimulated in a high-density environment as a consequence of higher demand for manoeuvrability and increased cognitive demands. We also show that the relative size of telencephalon and cerebellum changes with body size, which could be an effect of smoltification in larger fish. Overall, the results add to previous evidence showing that there is substantial brain growth plasticity in fish depending on the surrounding environment and developmental stage. Results also suggest that increased post-release performance of salmon reared at the lower density may not be due to larger total brain size or larger cerebellum or telencephalon size.

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Data accessibility statement

Data for the experiment are available from the figshare database <http://dx.doi.org/10.6084/m9.figshare.3750675>.

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