Early enrichment effects on brain development in hatchery-reared Atlantic salmon (*Salmo salar*): no evidence for a critical period

Joacim Näslund, Kim Aarestrup, Søren T. Thomassen, and Jörgen I. Johnsson

Abstract: In hatcheries, fish are normally reared in barren environments, which have been reported to affect their phenotypic development compared with wild conspecifics. In this study, Atlantic salmon (*Salmo salar*) alevins were reared in conventional barren hatchery trays or in either of two types of structurally enriched trays. We show that increased structural complexity during early rearing increased brain size in all investigated brain substructures. However, these effects disappeared over time after transfer to barren tanks for external feeding. Parallel to the hatchery study, a group of salmon parr was released into nature and recaptured at smoltification. These stream-reared smolts developed smaller brains than the hatchery reared smolts, irrespective of initial enrichment treatment. These novel findings do not support the hypothesis that there is a critical early period determining the brain growth trajectory. In contrast, our results indicate that brain growth is plastic in relation to environment. In addition, we show allometric growth in brain substructures over juvenile development, which suggests that comparisons between groups of different body size should be made with caution. These results can aid the development of ecologically sound rearing methods for conservational fish-stocking programs.

Résumé : En écloserie, les poissons sont normalement élevés dans des milieux stériles qui, selon certains auteurs, seraient à l'origine de leur développement phénotypique différent de celui de leurs congénères vivant en milieu naturel. Des alevins vésiculés de saumon atlantique (*Salmo salar*) ont été élevés dans des plateaux d'écloserie stériles classiques ou dans l'un ou l'autre de deux types de plateaux plus complexes sur le plan structural. Nous démontrons qu'une plus grande complexité structurale au début de l'élevage se traduit par une taille plus grande pour toutes les sous-structures étudiées du cerveau. Ces effets s'estompent toutefois avec le temps après le transfert dans des bassins stériles pour fins d'alimentation externe. Parallèlement à l'étude en écloserie, un groupe de tacons de saumon a été libéré en milieu naturel puis recapturé au moment de la smoltification. Ces saumoneaux élevés en rivière ont développé de plus petits cerveaux que les saumoneaux élevés en écloserie et ce, quel que soit le traitement enrichi initial. Ces nouveaux résultats n'appuient pas l'hypothèse voulant qu'il existe, au début de la vie du saumon, une période déterminante de la trajectoire de croissance du cerveau. Nos résultats indiquent plutôt que la croissance du cerveau est un phénomène plastique en ce qui concerne le milieu. Nous démontrons en outre une croissance allométrique des sous-structures du cerveau durant le développement juvénile qui suggère que la comparaison de groupes de tailles corporelles différentes nécessite une certaine prudence. Ces résultats peuvent contribuer à la mise au point de méthodes d'élevage écologiques pour des programmes d'empoissonnement aux fins de conservation.

[Traduit par la Rédaction]

Introduction

Animals are reared in captivity for various purposes, such as meat production, pet trade, laboratory use, and stocking into nature. Fishes are included in all these categories, and lately there has been rising concerns regarding their captive welfare (Huntingford et al. 2006). Mass-produced fish (e.g., for meat production and conservational rearing) are commonly reared in barren environments deprived of structural objects, chiefly to simplify hatchery routines and reduce costs. Such barren environments have, however, repeatedly been shown to have negative impact on behavioural and (or) neural development in animals, first described in rodents (Hebb 1947, reviewed by Rosenzweig and Bennett 1996) and lately also in different fish species (Álvarez and Nicieza 2003; Kihslinger et al. 2006; Burns et al. 2009). At first this deviation from the wild type was mainly attributed to inadvertent selection pressures in the artificial environment (Price 1999), but recently it has been shown that effects of artificial rearing can result directly from altered phenotypic reaction

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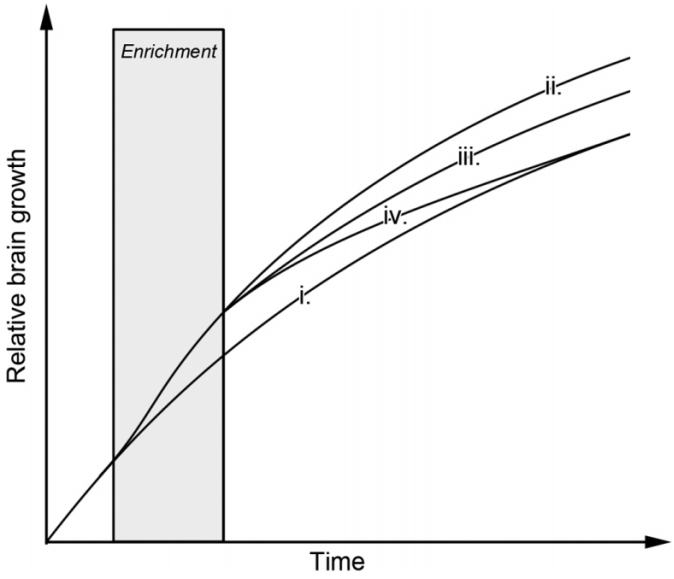
norms in captivity (Kihslinger et al. 2006; Burns et al. 2009; Mayer et al. 2011). Differences between wild and artificially reared fish include anatomical–morphological (Fleming et al. 1994, 1996; Vehanen and Huusko 2011) and physiological traits (Johnsson et al. 2001; Fleming et al. 2002), as well as behavioural (Berejikian 1995; Fleming and Einum 1997; Johnsson et al. 2001) and ecological traits (Vehanen et al. 2009). In nature, this can translate into maladaptive behaviours and reduced fitness (Fleming et al. 2000; Biro et al. 2004; Larsson et al. 2011). Divergence from natural behaviour also poses a potentially great problem when using artificially reared fish in basic research programs with the aim to gain knowledge about natural behaviour.

Several recent studies have focused on the brain development of captive reared fish (e.g., Kihslinger et al. 2006; Kihslinger and Nevitt 2006; Mayer et al. 2011). The overall brain size of an animal is generally thought to affect its cognitive ability (Lefebvre and Sol 2008), but this assumption still has to be substantiated further (Healy and Rowe 2007). The substructures of the brain are multifunctional to a certain degree, but their gross relative size generally corresponds to the ecological niche of the animal, which is especially evident in fish (Evans 1940; Shumway 2008). Therefore, the size of a substructure would likely reflect the relative importance of the senses or behavioural traits that it controls (Kotrschal et al. 1998; Ito et al. 2007). Several studies correspondingly show that the sizes of specific brain areas are under selection, both between and within species, which suggests that even small size changes can have biologically relevant effects (e.g., Kolm et al. 2009; Gonzalez-Voyer and Kolm 2010).

The correlations between brain size and behaviour are extensively researched in mammals and birds (Lefebvre and Sol 2008), and overall brain measurements have provided predictive neuroecological hypotheses in these taxa (Sherry 2006). Fish has been less studied, but some studies show correlational relationship between the size of certain brain structures and behaviour within a single species (Burns and Rodd 2008; Park and Bell 2010; Wilson and McLaughlin 2010). In contrast with mammals and birds, both the body and the brain show continuous growth through adulthood in fish (Zupanc 2006). This could allow for a flexible brain development that is not possible in taxa with only juvenile structural body growth, giving fishes a wider scope of plastic allocation of energy during ontogeny. Although environmental deprivation during juvenile life could lead to smaller brain size, and thereby potentially limited behavioural repertoire, this might be compensated quickly after release into more stimulating environment.

Environmental enrichment has been suggested as a means to improve the biological functioning of animals held in captivity (Newberry 1995; Brown and Day 2002). Enrichment effects on brain and behaviour are well studied in mammals, showing clear evidence of increased neurogenesis in rodents and less stereotypic behaviour in a wide range of animals (Hebb 1947; van Praag et al. 2000; Shyne 2006). Several recent studies show neural, behavioural, and cognitive effects in fish as well (Braithwaite and Salvanes 2005; Kihslinger and Nevitt 2006; Spence et al. 2011). However, other studies show that effects can be equivocal or lacking (Brockmark et al. 2007, 2010; Brydges and Braithwaite 2009), and certain enrichment types may cause distress (Fairhurst et al. 2011).

Fig. 1. Hypothetical brain growth trajectories resulting from enrichment in comparison with development in a non-enriched environment (trajectory *i*). Trajectory *ii* is offset by enrichment during a critical period in early life, increasing the relative brain size throughout life compared with the simple trajectory as proposed by Kihslinger and Nevitt (2006). Trajectory *iii* maintains the initial effect of enrichment but conforms to the curvature of the simple trajectory after enrichment is ended. In trajectory *iv*, brain growth decreases after enrichment is ended until it converges to the level of the simple trajectory. Elevation of trajectories is only conceptual.



These variable results may not be surprising considering the wide range of enrichment treatments and species used in different studies. Moreover, in some cases enrichment effects might only be detectable in the long term or with very large samples if cognitive challenges are rare. Nevertheless, environmental enrichment is still a promising method for conditioning fish for a more natural behaviour (Salvanes and Braithwaite 2005; Roberts et al. 2011; Rodewald et al. 2011).

In this study, we investigated how two different structural enrichments affect brain development in Atlantic salmon (*Salmo salar*) alevins and whether such early enrichment is enough to affect the brain growth trajectory also at later stages when structures are removed (a hypothesis proposed by Kihslinger and Nevitt 2006; Fig. 1). Furthermore, we released a batch of hatchery fish into a natural stream for half a year to investigate if the brain growth of these fish deviated from hatchery fish at smoltification. We also studied the pattern of early brain growth allometry to provide guidance for future studies of salmonid brain growth.

Materials and methods

Rearing

The subject species of this study was Atlantic salmon produced by artificial fertilization using 17 females and 30 males of River Storå stock, Denmark, in December 2007. The fish were reared at The Danish Centre for Wild Salmon hatchery in Randers, Denmark. Water was provided from a recirculating system with temperature matching the natural cycle. Dry food (Aller Performa, Aller Aqua, Denmark) was portioned out continuously at saturation levels using belt–clock feeders

Fig. 2. Pictures of salmon eggs in the initial rearing environment where they were hatched and spent their alevin life stage: (*a*) barren, standard hatchery environment (BA); (*b*) artificial substrate grid enrichment (EG); (*c*) stone enrichment (ES).

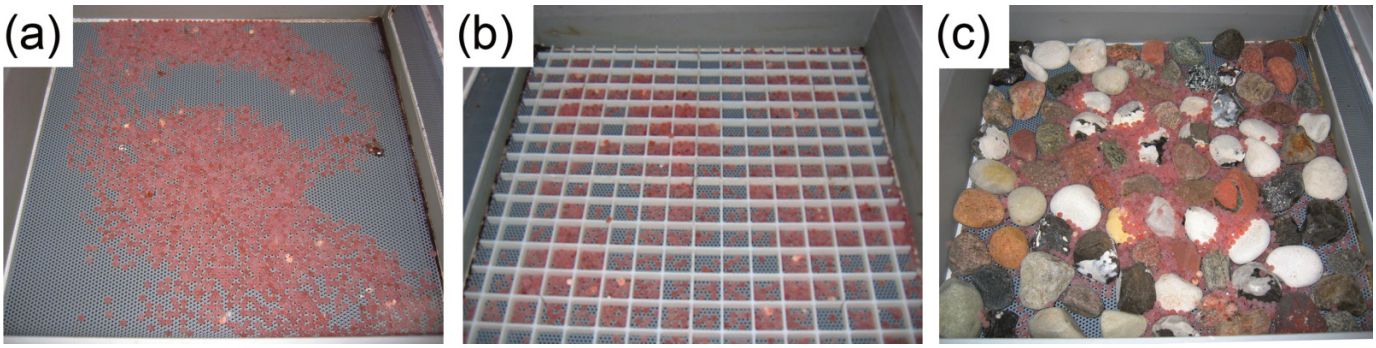


Table 1. Summary of the hatchery experimental design.

Tank dimension (cm)	Depth (cm)	Replicates per treatment	Environment	Initial number (individuals (SE))	Mortality	Temperature range (°C)	Water velocity ($L \cdot min^{-1}$)
Egg–alevin (2 Feb. – 14 May)							
40×40	7	5	—	2000[a]	—[b]	3.9–11.5	12–16
Fry (14 May – 9 Sept.)							
100×100	15	3	BA	3083 (63)	1577 (23)	7.5–14.8	12–16
			EG	3273 (192)	1435 (133)		
			ES	2966 (182)	1129 (206)		
Parr–smolt (9 Sept. – 1 April)							
200×200	30	1	BA	2948[a]	—[b]	No data	20–40
			EG	3580[a]	—[b]		
			ES	3620[a]	—[b]		

Note: BA, barren, standard hatchery environment; EG, artificial substrate grid enrichment; ES, stone enrichment; SE, standard error.
[a]Absolute numbers.
[b]No mortality recorded.

during light hours (24 h light between start feeding and July, thereafter natural light regime). The fish was subjected to any of three environments from the egg stage (2 February 2008) to the late alevin (i.e., yolk-sac fry) stage (7 May 2008). Average hatching date was 30 March. The different environments consisted of (*i*) the standard barren hatchery trays (BA; Fig. 2*a*), widely used in salmon hatcheries, (*ii*) artificial plastic substrate grid (EG) on the tray bottom (38 cm × 38 cm with partitions of 2.2 cm × 2.2 cm; Fig. 2*b*), or (*iii*) scattered stones (ES), approximately 4–5 cm in diameter, 1 stone per 10 cm^2 (Fig. 2*c*). Following this initial treatment, the fish were continuously kept in standard barren hatchery tanks. During the parr stage, the fish were pooled into three separate tanks only because of space constraints in the facility. Further details regarding rearing conditions are found in Table 1.

Natural release

On 2 October 2008, 1500 fish from each treatment were batch marked by fin clipping (adipose fin or left or right pelvic fin) and released into the nearby natural stream Villestrup Å. Released fish were spread approximately 7–9 km upstream from the river outlet in Mariager Fjord, eastern Denmark. The stream contains no natural Atlantic salmon, but has a strong sea-run brown trout (*Salmo trutta*) population.

Sampling

Samples were taken at four occasions in the hatchery during the rearing period: (*i*) late alevin stage (7 May 2008, end of environmental treatment; $n = 5 \times 5$), (*ii*) fry stage (13 June 2008; $n = 3 \times 5$, one less fish in one of the BA replicates), (*iii*) parr stage (1 October 2008 in connection with release of fish into nature; $n = 20$ for BA and EG; $n = 19$ for ES), and (*iv*) smolt stage (31 March – 1 April 2009). Included in the fourth sampling were the fish released into Villestrup Å, collected between 26 March and 14 May 2009 at smolt stage using a smolt trap ($n = 32$, including BA = 10, EG = 10, and ES = 12) and fish from the hatchery ($n = 30$, including BA = 10, EG = 10, and ES = 10). Equal numbers of fish were taken from each replicate tank, where applicable. At each sampling, total length of each fish was recorded. All fish sampled were killed by overdose of MS-222 (tricaine methanesulfonate, 10 $mg \cdot L^{-1}$).

Neural analysis

Heads were fixed and stored in phosphate-buffered 4% paraformaldehyde prior to dissection. After dissection the brains where photographed dorsally using a digital single lens reflex camera (Canon EOS 40D, Canon Inc., Japan) with a mounted super macro photo lens (Canon EF MP-E65/2.8 1-5X, Canon Inc.). The camera was mounted vertically

on a tripod at a fixed distance from the object brain. Brains where kept in saline water (6‰–6.6‰) during photography, and the level of this water was adjusted to the uppermost parts of the optic tectum whereby the brain adjusted itself horizontally with help of the surface tension in a standardized way. Illumination for photography was provided by two slave camera flashes. Pictures for each brain were shot in duplicates, and the brain was horizontally rotated 180 degrees and realigned between the shots.

The area of the whole brain and the dorsally visible substructures (olfactory bulbs, OB; telencephalon, TE; optic tectum, OT; and cerebellum, CE; Fig. 3) were measured on the digital images using ImageJ 1.41 (Rasband 1997–2011). All measurements were taken from each of the duplicate images, to the nearest 0.001 mm^2. The mean area from these measurements was analysed. Because of complications in the dissection, a few brains were missing one of the paired olfactory bulbs. In these cases the area of OB was calculated as twice the area of the remaining bulb.

We used dorsal area of four brain substructures as a proxy for size. A dorsal area estimate lacks the third dimension of the structure but contains the information about shape in the two dimensions used, in contrast with idealized ellipsoid models based on one-dimensional measurements of length, width, and height of substructures (Pollen et al. 2007; Shumway 2008). Repeatability of area measurement on the images was controlled for a subset of the alevin samples ($n = 15$) and was found to be excellent (overall Pearson correlation factor $R = 0.998$).

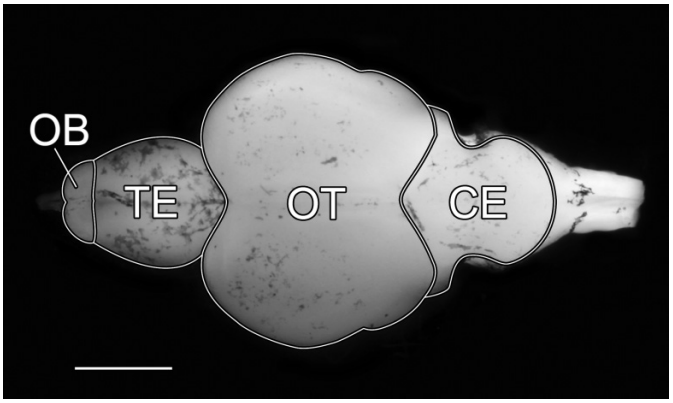
In addition to the experimental data, we also descriptively compared allometries in brain growth between the sampling periods using the pooled data from each sampling occurrence.

To control how well the dorsal area measurements correlated with brain size, we weighed the brains (cut off at the posterior brain stem) from the second sampling to the nearest 0.01 mg. The person taking measurements was blind to treatment.

Statistical analysis

The brain area measurements were logarithmically transformed ($\log_e$(area + 1)) and analysed using analysis of covariance (ANCOVA) models for whole brain size and multivariate analysis of covariance (MANCOVA) models for the substructures. When analysing the alevin and fry samples, total length was used as covariate to eliminate effects of body size, and the replicate tank was nested within treatment as a fixed factor. Homogeneity of slopes was violated in the fry analysis, mainly because smaller-sized fish from ES had relatively large optic tecta in comparison with fish from EG (based on visual inspection of slopes). This violation does not affect the main conclusions of the study. The brains from the parr and smolt sampling were analysed in the same way but without the nested factor because of lack of replicate tanks; slopes were homogenous. The smolt sampling occurred at the time of peak smoltification, when comparisons were made between the hatchery-raised fish and the fish released 6 months earlier into the natural stream. For this comparison, the hatchery-raised fish from the three treatments were pooled because of the lack of residual effects from the initial treatment at this stage. Treatment differences in relative dorsal substructure areas were analysed using individual analysis of variance (ANOVA) models. The assumption of homoscedasticity was analysed using Levene's test, and differences were found to be nonsignificant ($p > 0.05$) in all but one case (optic tecta in the fry sampling, $p = 0.043$). Normality of the data was tested with Shapiro–Wilk test; for the alevin and the fry data, we tested each replicate tank; for parr and smolt data, we tested each treatment. After Bonferroni correction for multiple comparisons, no significant deviations from normality were detected. All analyses were made using SPSS 17.0 for Windows (SPSS Inc., Chicago, Illinois).

Fig. 3. Schematic picture of brain areas measured in Atlantic salmon juveniles, dorsal view, anterior parts to the left. OB, olfactory bulbs; TE, telencephalon; OT, optic tectum; CE, cerebellum. Scale bar = 1 mm.



Results

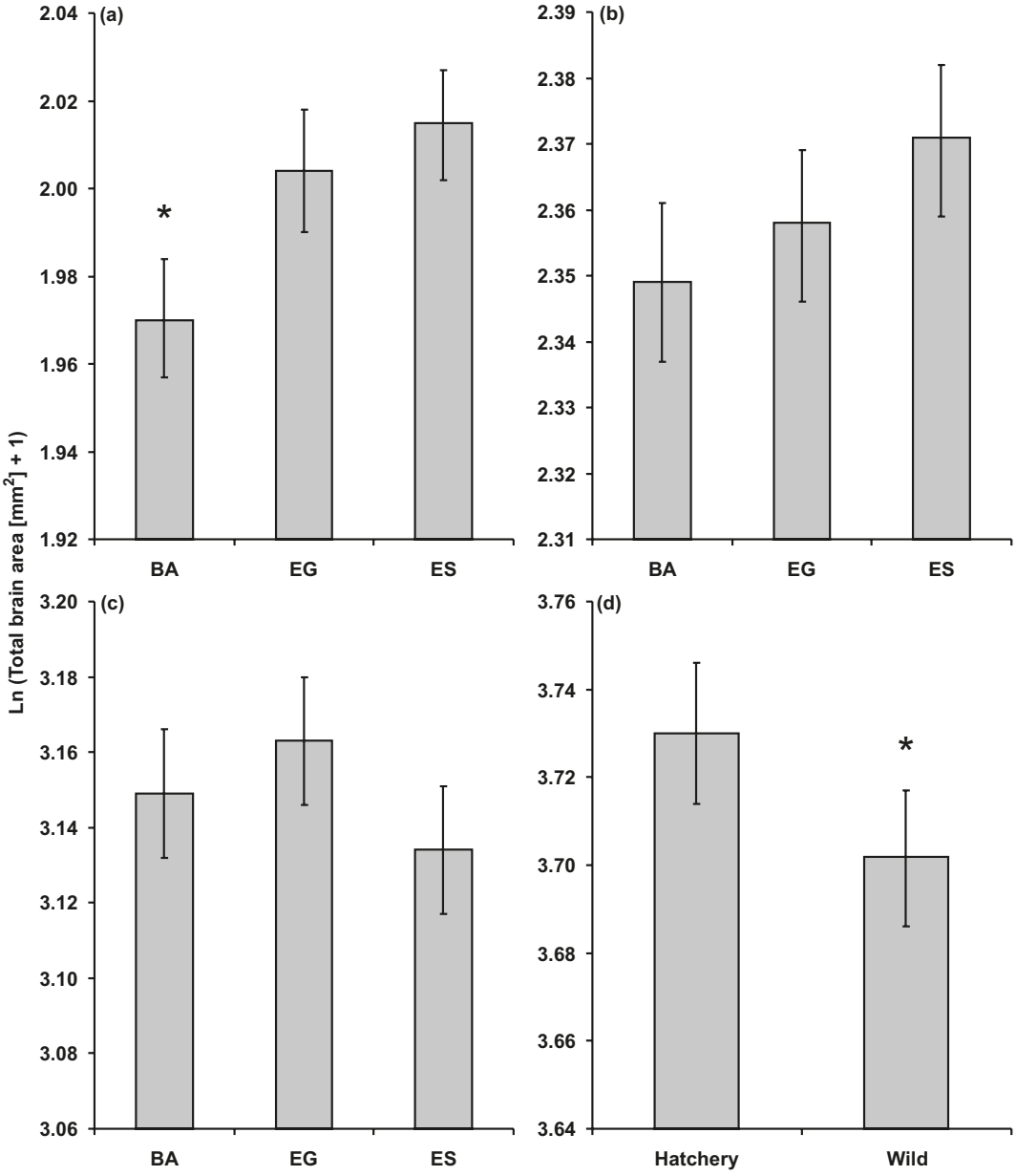
Body size

There was a significant effect of treatment on total body length at the alevin sampling, immediately after the initial enrichment, where the fishes subjected to artificial structure (EG) were on average 2.8% and 3.5% longer than fishes from stone (ES) and barren (BA) treatment, respectively (ANOVA: $F_{[2,60]} = 15.857$; $p < 0.001$). No statistical difference between ES and BA treatments could be detected. After the alevin sampling, no effects of treatment were detected on body length within age classes in hatchery fish (all $p > 0.2$). At the last sampling the stream-run smolts were on average 2% longer than hatchery smolts (ANOVA: $F_{[1,60]} = 48.614$; $p < 0.001$).

Brain size, alevin stage

At the first sampling, after the initial environmental treatment, there were significant positive enrichment effects on total dorsal brain area (ANCOVA: $F_{[2,59]} = 13.303$; $p < 0.001$; Fig. 4*a*). Sidak-adjusted estimated marginal means showed that the BA treatment produced significantly smaller ($p < 0.01$) brain sizes than both enrichment treatments; on average 3.9% smaller brains than EG treatment and 5.1% smaller than ES treatment (mean (95% CI): BA = 6.17 mm^2 (6.08–6.27 mm^2); EG = 6.42 mm^2 (6.32–6.52 mm^2); ES = 6.50 mm^2 (6.40–6.59 mm^2); covariate evaluated at 28.30 mm, all values are back-transformed). Multivariate analysis (MANCOVA) showed that this size effect could be significantly detected in all investigated substructures (Table 2). All substructures show treatment effect sizes of similar pattern as the results of the whole brain analysis: BA < EG <

Fig. 4. Dorsal brain area at standardized body lengths after (*a*) the initial enrichment treatment at the alevin sampling, (*b*) fry sampling, (*c*) parr sampling, and (*d*) after release into the wild at the smolt sampling. Standardized body lengths: (*a*) 28.30 mm, (*b*) 36.15 mm, (*c*) 71.76 mm, and (*d*) 116.2 mm. BA, barren, standard hatchery environment; EG, artificial substrate grid enrichment; ES, stone enrichment. Asterisks denote significant differences ($p < 0.05$).



ES (mean size in mm^2, BA, EG, ES): OB: 0.111, 0.122, 0.124; TE: 0.853, 0.868, 0.896; OT: 4.270, 4.447, 4.468; CE: 0.919, 0.962, 0.992; covariate evaluated at 28.30 mm, all values are back-transformed).

Brain size, fry stage

The overall pattern in mean brain size between treatments remained at the second sampling, but no differences were significant (Fig. 4*b*). The MANCOVA model on substructures was also nonsignificant (Table 2).

Brain size, parr stage

No differences between treatments were seen in total dorsal area at the third sampling (5 months after enrichment; Fig. 4*c*). MANCOVA on substructures from the third sampling was not significant, although the individual ANCOVA model of OT showed that the fish from the EG treatment had significantly larger dorsal area compared with ES (Table 2).

Brain size, stream-run vs. hatchery smolts

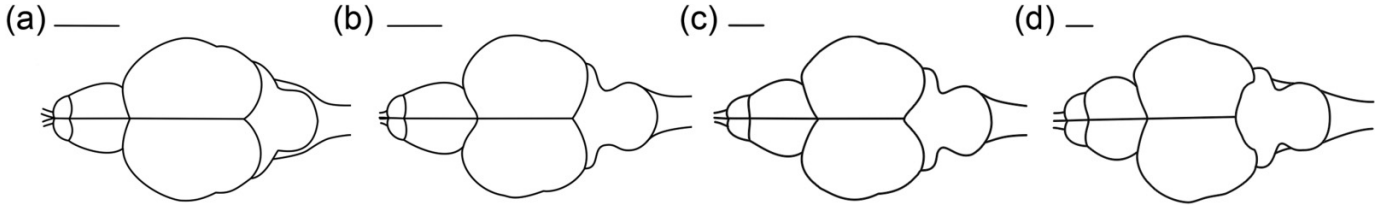
In total, 34 salmon were caught in the smolt trap in the stream Villestrup Å during spring 2009 (BA: $n = 10$; EG: $n = 11$; ES: $n = 13$). The statistical comparison between stream-run fish captured as running smolts and smolted hatchery fish showed that the fish living in the hatchery had on average 2.9% larger dorsal brain area (stream-run = 40.68 mm^2 (40.02–41.35 mm^2); hatchery = 39.53 mm^2 (38.88–40.14 mm^2); covariate evaluated at 116.2 mm; all val-

Table 2. Summary of multivariate analysis of covariance (MANCOVA) results for the alevin, fry, and parr sampling.

	Alevin				Fry				Parr	
	Treatment		Tank(treatment)		Treatment		Tank(treatment)		Treatment	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Whole model (Wilks' Λ)	4.76	<0.001*	0.49	0.998	0.97	0.471	1.01	0.512	1.12	0.354
Cerebellum	9.25	<0.001*	0.58	0.851	0.31	0.737	1.56	0.188	0.50	0.612
Optic tectum	10.40	<0.001*	0.38	0.966	1.58	0.220	1.07	0.401	4.03	0.023*
Telencephalon	7.41	0.001*	0.43	0.943	2.27	0.119	0.97	0.466	0.35	0.708
Olfactory bulbs	12.39	<0.001*	0.66	0.784	0.49	0.381	0.78	0.595	0.55	0.580

Note: All brain measures are ln(x + 1)-transformed. Asterisks (*) indicate significant results at the $p < 0.05$ level.

Fig. 5. Overview of allometric growth in juvenile Atlantic salmon with comparative schematic pictures from a fish close to mean length at each sampling, dorsal view, anterior part to the left: (*a*) alevin sampling (fish length = 29 mm); (*b*) fry sampling (fish length = 32 mm); (*c*) parr sampling (fish length = 72 mm); (*d*) smolt sampling (fish length = 116 mm). Scale bars = 1 mm.



ues are back-transformed) than fish released into the wild, based on Sidak-adjusted estimated marginal means corrected for body length (ANCOVA: $F_{[1,59]} = 4.873$; $p = 0.031$; Fig. 4*d*).

Validation of area measures

Correlation between brain mass and total dorsal area was high with a Pearson correlation factor of $R = 0.957$, suggesting that dorsal area is a good size estimate, as previously shown in guppies (*Poecilia reticulata*) by Burns with colleagues (Burns and Rodd 2008; Burns et al. 2009).

Allometries in brain growth

Different brain substructures investigated in this study clearly grew at different rates during ontogeny, as indicated by the difference in relative dorsal area for each substructure among the sampling periods (Fig. 5). The relative dorsal area of CE, TE, and OB increased with body length, but with an attenuating curve, while the opposite was true for OT (Figs. 6*a*–6*d*). There was a significant effect of treatment in the alevin sampling between BA and ES fishes in OB area relative to the total dorsal brain area (ANOVA: $F_{[2,72]} = 4.280$; $p = 0.018$). There was no evidence of treatment effects on relative size for TE, OT, or CE in the alevin sampling, neither was there evidence for any effects in fry, parr, or smolt sampling (all $p > 0.05$).

Discussion

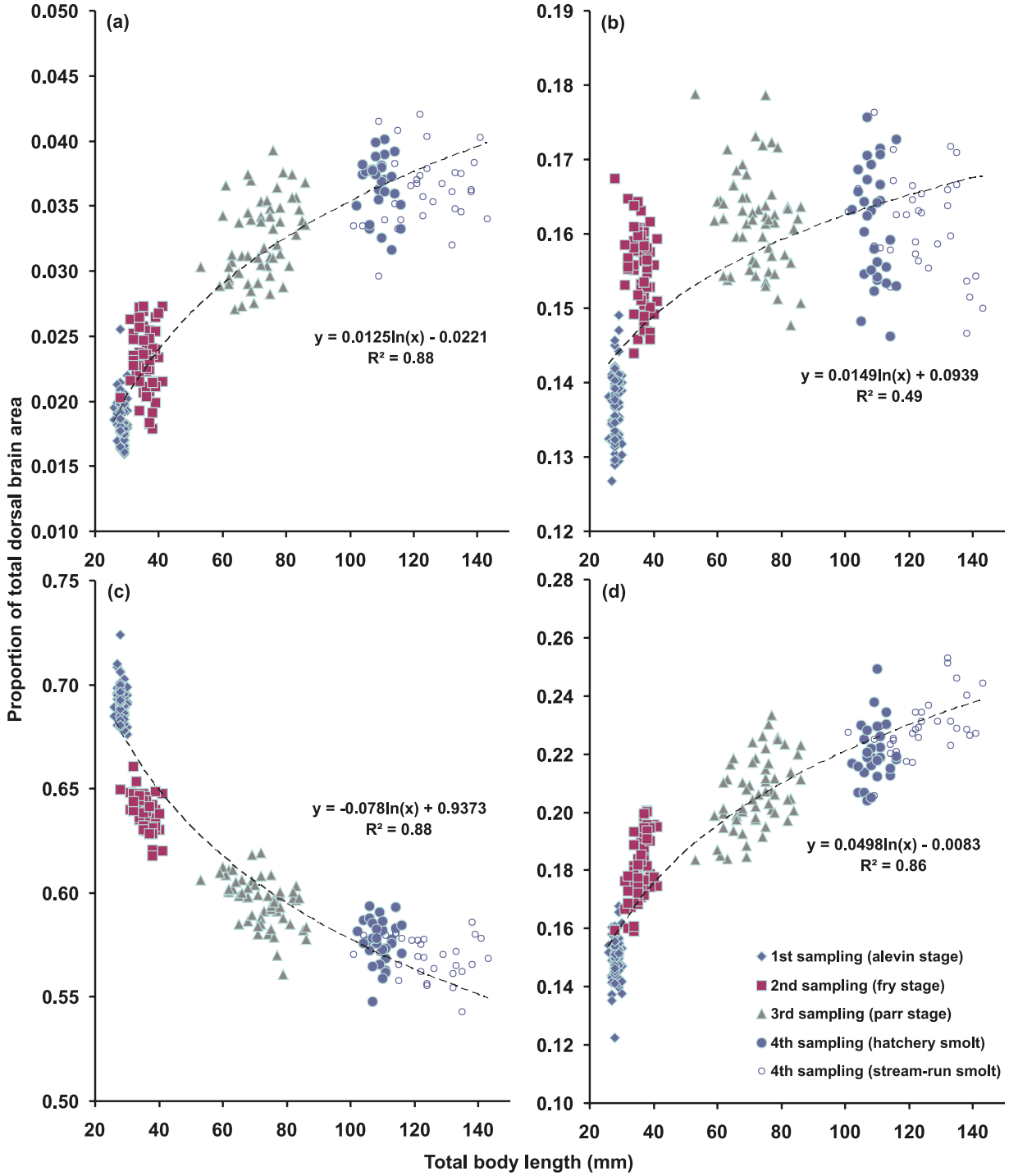
We found significant positive enrichment effects on overall brain size (all investigated substructures were larger) in Atlantic salmon at the alevin stage, immediately after the initial enrichment treatment. These effects subsequently disappeared, apparently at a gradual fashion, and at the parr sampling, after 3.5 months, the enrichment effects were absent without trace of initial effect pattern. The fourth sampling, 1 year after initial treatment, showed that fish stocked in a natural stream and recaptured as smolts had smaller brains than smolted fish from the same original batch kept in the hatchery during approximately the same time.

Our results are consistent with Kihslinger and Nevitt's (2006) observations of positive effects of early environmental enrichment on neural development in steelhead trout (i.e., sea-run rainbow trout, *Oncorhynchus mykiss*). Our results, however, are not concordant with their finding that effects could only be seen on the cerebellar substructure. We detected initial effects on the whole brain and all of the investigated substructures at the alevin stage, and the same pattern in mean sizes was found at the fry sampling, albeit nonsignificant. Our findings do not support the hypothesis of a permanently changed brain growth trajectory after initial enrichment during a critical period in early life (illustrated by trajectory *ii* in Fig. 1) as proposed by Kihslinger and Nevitt (2006). Instead, since no pattern from the initial effect is left at the parr stage, our study suggests that the growth rate of enriched fish levels out to the trajectory of the non-enriched fish (trajectory *iv* in Fig. 1) after the structural enrichment has been removed. Previous studies have mainly looked at brain size at one point in ontogeny, for instance after enrichment. In this study, we show that there is need for repeated sampling during the brain development to reveal whether initial enrichment effects on the neural system are maintained.

There are several accounts of behavioural differences between enriched and non-enriched fish. For instance, Atlantic salmon reared in enriched hatchery environment have been shown to reduce maladaptive risk-taking and increase the intake of natural food compared with standard hatchery salmon (Roberts et al. 2011; Rodewald et al. 2011). It is still not determined if these behavioural differences are linked to structural brain differences.

Much behavioural complexity can be achieved with quite few neurons in the central nervous system, as have been shown in insects (reviewed by Chittka and Niven 2009;

Fig. 6. Allometric dorsal area growth of brain substructures in relation to total brain dorsal area growth: (*a*) olfactory bulbs, (*b*) telencephalon, (*c*) optic tectum, (*d*) cerebellum. Regression line and formula show best logarithmic fit to the pooled data.



Burns et al. 2011). Larger brains could increase the potential for interconnectivity between neurons, the processing ability, and (or) the memory storage capacity but not necessarily cause a qualitatively different behaviour at any given time. Instead the bigger brains may result in increased capacity for information acquisition and in turn a more fail-safe system translating to quantitatively different behaviour over longer time periods, in the end making the fish less prone to fatal decision errors (Polani 2009). This scenario would be consistent with a growth–cognition trade-off in an environment where decision errors are not costly (Chittka et al. 2009). The effects of brain size on behaviour are debated, and causation should not be inferred from correlation alone (Bolhuis and Macphail 2001), but the evidence of high costs of brain maintenance suggests that an increased brain size would be maladaptive if there are no positive effects of it (Laughlin 2001). The smaller brains in non-enriched fish observed directly after environmental treatment in our study could thus be an effect of a growth–cognition trade-off producing different cognitive phenotypes in the differing environments (Tay-

lor et al. 2007). A barren environment probably poses less demand on the cognitive processing ability of the receiver (i.e., the fish) than a complex environment, since it contains less information (Shannon 1948). If this is the case, it could be adaptive to reduce the metabolic expenses put into brain growth and maintenance when it is not needed and instead allocate that energy to activity, growth of other bodily structures, and (or) antagonistic behaviour to ensure a high competitive ability against the companion fish. Höjesjö et al. (2004) showed that complexity in the environment reduces aggressiveness in brown trout, resulting in a decreased advantage to the more aggressive individuals. A recent study on pearl cichlids (*Geophagus brasiliensis*) also shows that environmental enrichment reduces aggression in aquaria (Kadry and Barreto 2010). Furthermore, Kihslinger and Nevitt (2006) showed that enriched steelhead alevins move less than those from standard hatchery environment. These results may indicate an energy trade-off between behaviour and other costly bodily processes, which could include brain size.

In addition to the positive effects of enrichment, we were also able to detect an apparent flexible nature of the brain growth later in life, as the stream-run fish had smaller brains than hatchery fish with the same origin. The environment present in the stocking stream, Villestrup Å, is certainly at least as complex in its structure, and thus in information content, as the stone treatment in the hatchery. Thus, it is surprising that the stream-run fish develop relatively smaller brains, especially since other studies show that wild fish have generally larger brains than hatchery conspecifics (e.g., Kihslinger et al. 2006; Burns et al. 2009; Mayer et al. 2011). This could be an indication that the relative brain size development is complex and dependent on several environmental factors, not only structural complexity. The smolts in the stream were significantly larger than the hatchery smolts, which indicates that growth was not limited. Instead there might have been a difference in energy allocation where stream-run fish allocate relatively more energy to structural growth than to neural growth compared with hatchery fish. Level of smoltification may also have influenced these results, as the wild fish were sampled over a longer time period. To the authors' knowledge, there are no studies on gross brain size changes in relation to smoltification, but at least smaller structural brain changes are known to occur (Ebbesson et al. 2011). The recapture rate in the natural stream was very low (<0.01%), but similar numbers were caught from each treatment, indicating similar numbers of smolting fish (as expected given that there were no significant differences between groups at release). Total smolt production or survival is hard to estimate given our data, since we did not capture resident fish, autumn-run smolts, or 2-year-old smolts in this study; the latter group is expected to constitute a large proportion of smolting fish in the geographical area.

Previous studies have shown seasonal changes in the size of brain structures in birds and amphibians (Nottebohm 1981; Takami and Urano 1984), probably linked to reproduction, and other studies have shown plasticity in the brain development in response to environmental factors (e.g., Sørensen et al. 2007; Ebbesson et al. 2011). However, to our knowledge there have yet been little indications of phenotypic flexibility (Piersma and Drent 2003) in brain growth of fish due to environmental factors. To entangle the phenotypic flexibility from the developmental plasticity is probably important for explaining many organismal adaptations in nature.

Ontogenetic allometries have previously been suggested to be able to influence results of analyses (Gonda et al. 2011). The present study found differences in relative growth rates of different brain areas during the juvenile development. The relative dorsal area of cerebellum, telencephalon, and olfactory bulbs increased with size during early ontogeny, while optic tectum decreased. The change appeared to level out between the third and fourth sampling, perhaps reflecting the final organisation of the brain in the adult fish. Allometries of this kind make the comparison between early size and (or) age classes of fish difficult. Thus, it is important to recognize and control for this when analysing the gross brain morphology of juvenile fish, especially if size differs systematically between treatments. However, this would have little effect in our study, since separate analyses were performed for each of the age classes.

There has been a long-term trend of declining populations of Atlantic salmon, both in Europe and North America, which to a large extent is due to human impact on stocks and their natural habitat (Parrish et al. 1998; Kallio-Nyberg et al. 2011; Piccolo et al. 2011). Stocking of hatchery-reared fish is one of the main actions taken to cope with and counterbalance this decline. It is therefore of economical, conservational, ecological, and ethical importance that the fish released are able to survive in nature (Armstrong and Seddon 2008; Piccolo et al. 2011). Release of unfit fish would not only be a waste of monetary resources and go against the IUCN guidelines for restocking, but also have effects on natural populations and ecosystems and lead to compromised welfare of the released fish (IUCN 1998; Brännäs and Johnsson 2008).

To summarize, we have presented results indicating a positive effect on neural development from environmental enrichment in Atlantic salmon. Furthermore, we found no evidence of an early critical period having lasting effects on brain development. Instead the effects of enrichment seem to gradually disappear after the treatment was terminated. Thus, we did not find any support for a critical period for setting the brain growth trajectory in salmon fry. We suggest that the brain growth trajectory is adaptively plastic during the life of a fish. There is still much work to be done regarding both causes and effects and their proximal and ultimate mechanisms in this field of fish biology. Further manipulative studies incorporating both brain analyses and other phenotypic effects would further widen the scope of current research and its applications in aquaculture and conservation biology.

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References

Álvarez, D., and Nicieza, A.G. 2003. Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and

domestication. J. Fish Biol. **63**(6): 1565–1577. doi:10.1111/j.1095-8649.2003.00267.x.

Armstrong, D.P., and Seddon, P.J. 2008. Directions in reintroduction biology. Trends Ecol. Evol. **23**(1): 20–25. doi:10.1016/j.tree.2007.10.003. PMID:18160175.

Berejikian, B. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. Can. J. Fish. Aquat. Sci. **52**(11): 2476–2482. doi:10.1139/f95-838.

Biro, P.A., Abrahams, M.V., Post, J.R., and Parkinson, E.A. 2004. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. Proc. Biol. Sci. **271**(1554): 2233–2237. doi:10.1098/rspb.2004.2861. PMID:15539348.

Bolhuis, J.J., and Macphail, E.M. 2001. A critique of the neuroecology of learning and memory. Trends Cogn. Sci. **5**(10): 426–433. doi:10.1016/S1364-6613(00)01753-8. PMID:11707381.

Braithwaite, V.A., and Salvanes, A.G.V. 2005. Environmental variability in the early rearing environment generates behaviourally flexible cod: implications for rehabilitating wild populations. Proc. Biol. Sci. **272**(1568): 1107–1113. doi:10.1098/rspb.2005.3062. PMID:16024371.

Brännäs, E., and Johnsson, J.I. 2008. Behaviour and welfare in farmed fish. *In* Fish behaviour. *Edited by* C. Magnhagen, V.A. Braithwaite, E. Forsgren, and B.G. Kapoor. Science Publishers, Enfield, N.H., USA. pp. 593–627.

Brockmark, S., Neregård, L., Bohlin, T., Björnsson, B.T., and Johnsson, J.I. 2007. Effects of rearing density and structural complexity on the pre- and postrelease performance of Atlantic salmon. Trans. Am. Fish. Soc. **136**(5): 1453–1462. doi:10.1577/T06-245.1.

Brockmark, S., Adriaenssens, B., and Johnsson, J.I. 2010. Less is more: density influences the development of behavioural life skills in trout. Proc. R. Soc. Lond. B Biol. Sci. **277**(1696): 3035–3043. doi:10.1098/rspb.2010.0561.

Brown, C., and Day, R.L. 2002. The future of stock enhancements: lessons for hatchery practice from conservation biology. Fish Fish. **3**(2): 79–94. doi:10.1046/j.1467-2979.2002.00077.x.

Brydges, N.M., and Braithwaite, V.A. 2009. Does environmental enrichment affect the behaviour of fish commonly used in laboratory work? Appl. Anim. Behav. Sci. **118**(3–4): 137–143. doi:10.1016/j.applanim.2009.02.017.

Burns, J.G., and Rodd, F.H. 2008. Hastiness, brain size and predation regime affect the performance of wild guppies in a spatial memory task. Anim. Behav. **76**(3): 911–922. doi:10.1016/j.anbehav.2008.02.017.

Burns, J.G., Saravanan, A., and Rodd, F.H. 2009. Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. Ethology, **115**(2): 122–133. doi:10.1111/j.1439-0310.2008.01585.x.

Burns, J.G., Foucaud, J., and Mery, F. 2011. Costs of memory: lessons from 'mini' brains. Proc. R. Soc. Lond. Ser. B Biol. Sci. **278**(1707): 885–888.

Chittka, L., and Niven, J. 2009. Are bigger brains better? Curr. Biol. **19**(21): R995–R1008. doi:10.1016/j.cub.2009.08.023. PMID:19922859.

Chittka, L., Skorupski, P., and Raine, N.E. 2009. Speed–accuracy tradeoffs in animal decision making. Trends Ecol. Evol. **24**(7): 400–407. doi:10.1016/j.tree.2009.02.010. PMID:19409649.

Ebbesson, L.O.E., Nilsen, T.O., Helvik, J.V., Tronci, V., and Stefansson, S.O. 2011. Corticotropin-releasing factor neurogenesis during midlife development in salmon: genetic, environmental and thyroid hormone regulation. J. Neuroendocrinol. **23**(8): 733–741. doi:10.1111/j.1365-2826.2011.02164.x. PMID:21592238.

Evans, H.M. 1940. Brain and body of fish: a study of brain pattern in relation to hunting and feeding in fish. The Blakiston Company, Philadelphia, Pa.

Fairhurst, G.D., Frey, M.D., Reichert, J.F., Szelest, I., Kelly, D.M., and Bortolotti, G.R. 2011. Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. PLoS ONE, **6**(3): e17663. doi:10.1371/journal.pone.0017663. PMID:21412426.

Fleming, I.A., and Einum, S. 1997. Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. ICES J. Mar. Sci. **54**(6): 1051–1063. doi:10.1016/S1054-3139(97)80009-4.

Fleming, I.A., Jonsson, B., and Gross, M.R. 1994. Phenotypic divergence of sea-ranched, farmed, and wild salmon. Can. J. Fish. Aquat. Sci. **51**(12): 2808–2824. doi:10.1139/f94-280.

Fleming, I.A., Jonsson, B., Gross, M.R., and Lamberg, A. 1996. An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). J. Appl. Ecol. **33**(4): 893–905. doi:10.2307/2404960.

Fleming, I.A., Hindar, K., Mjølnerød, I.B., Jonsson, B., Balstad, T., and Lamberg, A. 2000. Lifetime success and interactions of farm salmon invading a native population. Proc. Biol. Sci. **267**(1452): 1517–1523. doi:10.1098/rspb.2000.1173. PMID:11007327.

Fleming, I.A., Agustsson, T., Finstad, B., Johnsson, J.I., and Björnsson, B.T. 2002. Effects of domestication on growth physiology and endocrinology of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **59**(8): 1323–1330. doi:10.1139/f02-082.

Gonda, A., Herczeg, G., and Merilä, J. 2011. Population variation in brain size of nine-spined sticklebacks (*Pungitius pungitius*) — local adaptation or environmentally induced variation? BMC Evol. Biol. **11**(75): 1–11. doi:10.1186/1471-2148-11-75. PMID:21194491.

Gonzalez-Voyer, A., and Kolm, N. 2010. Sex, ecology and the brain: evolutionary correlates of brain structure volumes in Tanganyikan cichlids. PLoS ONE, **5**(12): e14355. doi:10.1371/journal.pone.0014355. PMID:21179407.

Healy, S.D., and Rowe, C. 2007. A critique of comparative studies of brain size. Proc. Biol. Sci. **274**(1609): 453–464. doi:10.1098/rspb.2006.3748. PMID:17476764.

Hebb, D.O. 1947. The effects of early experience on problem solving at maturity. Am. Psychol. **2**: 306–307.

Höjesjö, J., Johnsson, J., and Bohlin, T. 2004. Habitat complexity reduces the growth of aggressive and dominant brown trout (*Salmo trutta*) relative to subordinates. Behav. Ecol. Sociobiol. **56**(3): 286–289. doi:10.1007/s00265-004-0784-7.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P., and Turnbull, J.F. 2006. Current issues in fish welfare. J. Fish Biol. **68**(2): 332–372. doi:10.1111/j.0022-1112.2006.001046.x.

Ito, H., Ishikawa, Y., Yoshimoto, M., and Yamamoto, N. 2007. Diversity of brain morphology in teleosts: brain and ecological niche. Brain Behav. Evol. **69**(2): 76–86. doi:10.1159/000095196. PMID:17230015.

IUCN. 1998. IUCN Guidelines for re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group Gland, Switzerland and Cambridge, UK.

Johnsson, J.I., Höjesjö, J., and Fleming, I.A. 2001. Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. Can. J. Fish. Aquat. Sci. **58**(4): 788–794. doi:10.1139/f01-025.

Kadry, V.O., and Barreto, R.E. 2010. Environmental enrichment reduces aggression of pearl cichlid, *Geophagus brasiliensis*, during resident–intruder interactions. Neotrop. Ichthyol. **8**(2): 329–332. doi:10.1590/S1679-62252010000200011.

Kallio-Nyberg, I., Salminen, M., Saloniemi, I., and Lindroos, M.

2011. Effects of marine survival, precocity and other life history traits on the cost–benefit of stocking salmon in the Baltic Sea. Fish. Res. **110**(1): 111–119. doi:10.1016/j.fishres.2011.03.019.

Kihslinger, R.L., and Nevitt, G.A. 2006. Early rearing environment impacts cerebellar growth in juvenile salmon. J. Exp. Biol. **209**(3): 504–509. doi:10.1242/jeb.02019. PMID:16424100.

Kihslinger, R.L., Lema, S.C., and Nevitt, G.A. 2006. Environmental rearing conditions produce forebrain differences in wild Chinook salmon *Oncorhynchus tshawytscha*. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. **145**(2): 145–151. doi:10.1016/j.cbpa.2006.06.041.

Kolm, N., Gonzalez-Voyer, A., Brelin, D., and Winberg, S. 2009. Evidence for small scale variation in the vertebrate brain: mating strategy and sex affect brain size and structure in wild brown trout (*Salmo trutta*). J. Evol. Biol. **22**(12): 2524–2531. doi:10.1111/j.1420-9101.2009.01875.x. PMID:19878498.

Kotrschal, K., van Staaden, M.J., and Huber, R. 1998. Fish brains: evolution and environmental relationships. Rev. Fish Biol. Fish. **8**(4): 373–408. doi:10.1023/A:1008839605380.

Larsson, S., Linnansaari, T., Vatanen, S., Serrano, I., and Haikonen, A. 2011. Feeding of wild and hatchery reared Atlantic salmon (*Salmo salar* L.) smolts during downstream migration. Environ. Biol. Fishes, **92**(3): 361–369. doi:10.1007/s10641-011-9846-7.

Laughlin, S.B. 2001. Energy as a constraint on the coding and processing of sensory information. Curr. Opin. Neurobiol. **11**(4): 475–480. doi:10.1016/S0959-4388(00)00237-3. PMID:11502395.

Lefebvre, L., and Sol, D. 2008. Brains, lifestyles and cognition: Are there general trends? Brain Behav. Evol. **72**(2): 135–144. doi:10.1159/000151473. PMID:18836259.

Mayer, I., Meager, J., Skjæraasen, J., Rodewald, P., Sverdrup, G., and Fernö, A. 2011. Domestication causes rapid changes in heart and brain morphology in Atlantic cod (*Gadus morhua*). Environ. Biol. Fishes, **92**(2): 181–186. doi:10.1007/s10641-011-9831-1.

Newberry, R.C. 1995. Environmental enrichment: Increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. **44**(2–4): 229–243. doi:10.1016/0168-1591(95)00616-Z.

Nottebohm, F. 1981. A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. Science, **214**(4527): 1368–1370. doi:10.1126/science.7313697. PMID:7313697.

Park, P.J., and Bell, M.A. 2010. Variation of telencephalon morphology of the threespine stickleback (*Gasterosteus aculeatus*) in relation to inferred ecology. J. Evol. Biol. **23**(6): 1261–1277. doi:10.1111/j.1420-9101.2010.01987.x. PMID:20406344.

Parrish, D.L., Behnke, R.J., Gephard, S.R., McCormick, S.D., and Reeves, G.H. 1998. Why aren't there more Atlantic salmon (*Salmo salar*)? Can. J. Fish. Aquat. Sci. **55**(S1): 281–287. doi:10.1139/d98-012.

Piccolo, J.J., Norrgård, J.R., Greenberg, L.A., Schmitz, M., and Bergman, E. 2011. Conservation of endemic landlocked salmonids in regulated rivers: a case-study from Lake Vänern, Sweden. Fish Fish. [In press.] doi:10.1111/j.1467-2979.2011.00437.x.

Piersma, T., and Drent, J. 2003. Phenotypic flexibility and the evolution of organismal design. Trends Ecol. Evol. **18**(5): 228–233. doi:10.1016/S0169-5347(03)00036-3.

Polani, D. 2009. Information: currency of life? HFSP J. **3**(5): 307–316. doi:10.2976/1.3171566. PMID:20357888.

Pollen, A.A., Dobberfuhl, A.P., Scace, J., Igulu, M.M., Renn, S.C.P., Shumway, C.A., and Hofmann, H.A. 2007. Environmental complexity and social organization sculpt the brain in Lake Tanganyikan cichlid fish. Brain Behav. Evol. **70**(1): 21–39. doi:10.1159/000101067. PMID:17389793.

Price, E.O. 1999. Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. **65**(3): 245–271. doi:10.1016/S0168-1591(99)00087-8.

Rasband, W. 1997–2011. ImageJ 1.41 [online]. Available from http://rsb.info.nih.gov/ij/.

Roberts, L.J., Taylor, J., and Garcia de Leaniz, C. 2011. Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation. Biol. Conserv. **144**(7): 1972–1979. doi:10.1016/j.biocon.2011.04.017.

Rodewald, P., Hyvärinen, P., and Hirvonen, H. 2011. Wild origin and enriched environment promote foraging rate and learning to forage on natural prey of captive reared Atlantic salmon parr. Ecol. Freshw. Fish, **20**(4): 569–579. doi:10.1111/j.1600-0633.2011.00505.x.

Rosenzweig, M.R., and Bennett, E.L. 1996. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav. Brain Res. **78**(1): 57–65. doi:10.1016/0166-4328(95)00216-2. PMID:8793038.

Salvanes, A., and Braithwaite, V. 2005. Exposure to variable spatial information in the early rearing environment generates asymmetries in social interactions in cod (*Gadus morhua*). Behav. Ecol. Sociobiol. **59**(2): 250–257. doi:10.1007/s00265-005-0031-x.

Shannon, C.E. 1948. A mathematical theory of communication. Bell Syst. Tech. J. **27**(3): 379–423.

Sherry, D.F. 2006. Neuroecology. Annu. Rev. Psychol. **57**(1): 167–197. doi:10.1146/annurev.psych.56.091103.070324. PMID:16318593.

Shumway, C.A. 2008. Habitat complexity, brain and behavior. Brain Behav. Evol. **72**(2): 123–134. doi:10.1159/000151472. PMID:18836258.

Shyne, A. 2006. Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. Zoo Biol. **25**(4): 317–337. doi:10.1002/zoo.20091.

Sørensen, C., Øverli, Ø., Summers, C.H., and Nilsson, G.E. 2007. Social regulation of neurogenesis in teleosts. Brain Behav. Evol. **70**(4): 239–246. doi:10.1159/000105487. PMID:17914255.

Spence, R., Magurran, A., and Smith, C. 2011. Spatial cognition in zebrafish: the role of strain and rearing environment. Anim. Cogn. **14**(4): 607–612. doi:10.1007/s10071-011-0391-8. PMID:21360117.

Takami, S., and Urano, A. 1984. The volume of the toad medial amygdala–anterior preoptic complex is sexually dimorphic and seasonally variable. Neurosci. Lett. **44**(3): 253–258. doi:10.1016/0304-3940(84)90031-4. PMID:6728295.

Taylor, S.F., Tishby, N., and Bialek, W. 2007. Information and fitness. Quantitative Biology > Populations and Evolution, arXiv:0712.4382v1 [q-bio.PE].

van Praag, H., Kempermann, G., and Gage, F.H. 2000. Neural consequences of environmental enrichment. Nat. Rev. Neurosci. **1**(3): 191–198. doi:10.1038/35044558. PMID:11257907.

Vehanen, T., and Huusko, A. 2011. Brown trout *Salmo trutta* express different morphometrics due to divergence in the rearing environment. J. Fish Biol. **79**(5): 1167–1181. doi:10.1111/j.1095-8649.2011.03093.x. PMID:22026600.

Vehanen, T., Huusko, A., and Hokki, R. 2009. Competition between hatchery-raised and wild brown trout *Salmo trutta* in — do hatchery releases have negative effects on wild populations? Ecol. Freshw. Fish, **18**(2): 261–268. doi:10.1111/j.1600-0633.2008.00343.x.

Wilson, A., and McLaughlin, R. 2010. Foraging behaviour and brain morphology in recently emerged brook charr, *Salvelinus fontinalis*. Behav. Ecol. Sociobiol. **64**(11): 1905–1914. doi:10.1007/s00265-010-1002-4.

Zupanc, G. 2006. Neurogenesis and neuronal regeneration in the adult fish brain. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. **192**(6): 649–670. doi:10.1007/s00359-006-0104-y. PMID:16463148.