Norwegian University of Life Sciences
Faculty of Veterinary Medicine

Philosophiae Doctor (PhD)
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Effects of the hatchery environment on neurobiology and behaviour in Atlantic salmon: implications for stocking

Effekter av oppvekstmiljø på nevrobiologi og atferd hos klekkerilaks: implikasjoner for kultivering

Daan Mes

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Summary

The Atlantic salmon (*Salmo salar*) is an iconic fish species with a widespread historic abundance, but recent decades have witnessed a dramatic decline in wild stocks due to a variety of anthropogenic factors, especially overfishing and loss of habitat. To mitigate the impacts of these anthropogenic effects, millions of hatchery-reared Atlantic salmon are released yearly into rivers through stocking programs, which aim to augment the productivity of wild populations. However, these stocked fish are reared under uniform and stimulus-poor hatchery conditions and consequently, they are behaviourally naïve at time of release. For example, hatchery-reared salmonids often show impaired foraging and antipredator behaviour compared to wild conspecifics, which contributes to the observed high post-release mortality rates in stocked fish. Although the effects of the hatchery environment on fish behaviour are relatively well described, the brain, which is the key organ that translates environmental stimuli into appropriate behavioural responses, remains gravely understudied. The few studies which have investigated the impact of the hatchery environment on the fish central nervous system have mostly mapped the expression of neuroplasticity and neurogenesis genes in the entire brain, or large brain structures, such as the whole telencephalon. However, the brain is a complex organ, composed of a plethora of neural subpopulations, each with distinct functionalities and characteristics. When quantifying whole-brain levels of neuroplasticity markers, one studies a conglomerate of many different neural subregions, and regional differences can therefore not be detected. The aim of this thesis is to gain a better insight into the neural differences between wild and hatchery-reared fish, specifically within neural subpopulations of the telencephalon, and how innovative hatchery protocols can improve the neurobiology, behaviour and post-release survival of hatchery-reared salmon.

First, we made a detailed characterisation of the neurobiology of juvenile wild and hatchery-reared Atlantic salmon parr. This was achieved by quantifying the expression of the neuroplasticity marker *brain-derived neurotrophic factor* (*bdnf*) and the neural activity marker *cfos* in five neural populations within the telencephalon of wild and hatchery-reared juvenile salmon under both basal and acute-stress conditions (**Paper I**). We found that expression of *bdnf* and *cfos* varied greatly between the studied telencephalic subregions, confirming that these subregions have a distinct responsiveness to environmental stimuli. Compared to wild fish, hatchery-reared fish of the same genetic origin showed higher post-stress neural activation in the ventral area of the dorsolateral pallium (Dlv), which is an important brain region associated with relational memory and spatial orientation.

Furthermore, wild fish displayed stress-induced upregulation of *bdnf* in the dorsomedial pallium (Dm), which regulates emotional learning and stress reactivity, while this was not the case for hatchery-reared individuals. This study showed that targeting telencephalic subregions can reveal expression patterns that escape detection when studying the entire telencephalon as a whole. Moreover, we demonstrated that the hatchery environment affects neuroplasticity and neural activation in brain regions which are important for learning processes and stress reactivity, providing a neuronal foundation for the behavioural differences observed between wild and hatchery-reared fish.

After we had characterised neural differences in telencephalic subregions between wild and hatchery-reared salmon, we assessed whether structural environmental enrichment (EE) of the rearing environment could increase region-specific neural plasticity and stocking success in hatchery-reared salmon (**Paper II**). After seven weeks of treatment, EE-reared parr showed higher post-release freshwater survival rates compared to control individuals, which were reared in standard uniform hatchery tanks. This improved stocking performance did not, however, appear to be linked to significant changes in the expression of telencephalic plasticity markers.

Although structural EE has shown some, albeit inconsistent, beneficial effects on fish stocking success across studies, hatchery managers are reluctant to implement this measure in their hatcheries because of hygienic and operational limitations. Therefore, it is important to develop alternative rearing methods which can enhance fish neural development and are more practical to implement in the hatchery. One of these alternative rearing methods is swimming exercise, which has previously been linked to increased post-release survival in salmonids. As running exercise is associated with increased neural plasticity in mammals, we investigated in **Paper III** whether swimming exercise could serve as an alternative rearing strategy to promote Atlantic salmon neural plasticity and cognition. After eight weeks of sustained swimming, we found increased expression of neuroplasticity-related transcripts in the telencephalon transcriptome of exercised salmon. However, we did not find any evidence for increased cognition in exercised fish, in terms of their ability to solve a spatial orientation task in a maze test. While previous studies have reported positive physiological effects of swimming exercise, such as improved growth efficiency and stress reduction, this is the first time that exercise-enhanced neural plasticity has been reported in salmonids, building a case for exploring further the potential of implementing swimming exercise to improve the stocking success of reared salmonids.

In summary, the results presented in this thesis advance the field of applied fish neurobiology in a stocking context by characterising telencephalic neural plasticity markers in Atlantic salmon on a more detailed level than previously studied. We demonstrate that EE can improve juvenile salmon survival during freshwater residency, but that the effects of EE on neural plasticity are limited in the studied telencephalic regions. We identify swimming exercise as a promising novel tool to improve neural plasticity in salmon, and we remark that exercise has additional physiological benefits and is relatively easy to implement in hatcheries. We therefore suggest that future work should aim at validating the potential use of exercise in the optimisation of hatchery conditions for stocking programs, and that further research is needed to increase our understanding on the link between the rearing environment, the brain and behaviour.

Sammendrag

Laks (*Salmo salar*) er en ikonisk fiskeart som historisk sett har funnes i overflod, men som de siste tiår har opplevd en dramatisk nedgang i villpopulasjonen. Denne nedgangen skyldes hovedsakelig antropogene faktorer som overfisking og habitatinnskrenkning.
Som en motkraft til den minkende populasjon blir det gjennom kultiveringsprogrammer satt ut millioner av fisk fra klekkerier og ut i elvene årlig. Disse fiskene er imidlertid vokst opp under uniforme og stimuluslave omgivelser, noe som gjør dem atferdsmessig naive i møte med elven. For eksempel viser utsatt laks nedsatt forings- og antipredatorevne sammenlignet med villaks, noe som bidrar til høyere dødelighet hos denne gruppen. Selv om klekkerimiljøets effekter på atferd er relativt godt beskrevet, så er hjernen, selve hovedorganet som omsetter omgivelsenes stimuli til en passende atferd, fremdeles underbeskrevet. De få studiene som har undersøkt påvirkningen fra klekkerimiljøet på fiskens sentrale nervesystem har stort sett kartlagt uttrykket av gener involvert i nevroplastisitet og nevrogenese i enten hele hjernen eller større hjernestrukturer, som telencephalon. Hjernen er imidlertid et komplekst organ, sammensatt av et utall nevrale subpopulasjoner, hver med distinkte funksjonaliteter og karakteristikker som driver ulike atferder. Når man kvantifiserer markører for nevroplastisitet på helhjerne-nivå, så studerer man et konglomerat av ulike nevrale regioner på samme tid og kan dermed ikke detektere eventuelle regionale forskjeller. Hensikten bak denne avhandlingen var dels å bedre forståelsen av potensielle nevrale forskjeller mellom klekkeri- og villfisk, og dels å undersøke om innovative klekkeriprotokoller kan forbedre nevrobiologien, atferden og overlevelsen til kultivert laks etter elveutsettelsen.

Arbeidet startet med en detaljert karakterisering av nevrobiologien til kultivert og vill parr (juvenil laks). Dette ble oppnådd ved å kvantifisere genuttrykket av en nevroplastisitetsmarkør, *brain-derived neurotrophic factor* (*bdnf*), og en nevral aktivitetsmarkør, *cfos*, i fem ulike nevrale populasjoner innad i telencephalon til parr av klekkeri- og villaks under både basale- og akutte stress-tilstander (**Artikkel I**). Her fant vi at genuttrykket av *bdnf* og *cfos* varier sterkt mellom de ulike delene av telencephalon, noe som bekrefter at disse delene har distinkte responser til omgivelsene. Sammenlignet med villfisk har klekkerifisk, med det samme genetiske opphav som villfisken, høyere nevral aktivering etter stress i den ventrale delen av det dorsolaterale pallium (Dlv), et viktig område av hjernen assosiert med deklarativ hukommelse og romlig orientering. Videre hadde villfisken en stressindusert oppregulering av *bdnf* i det dorsomediale pallium (Dm), et område som

regulerer emosjonell læring og stressreaktivitet, mens dette ikke var tilfelle for klekkerifisken. Dette studiet illustrerte at å undersøke delregioner av telencephalon kan avsløre mønstre som blir maskert ved å undersøke telencephalon som en enhet. I tillegg ble det vist at klekkerimiljøet påvirker nevroplastisiteten og den nevrale aktiviteten i hjerneområder viktige for læreprosesser og stressreaktivitet, noe som gir et nevralt fundament for de atferdsmessige forskjeller som blir observert mellom vill og kultivert fisk.

Etter karakteriseringen av de nevrale forskjellene i telencephalons delregioner mellom vill og kultivert laks, undersøkte vi videre om strukturell berikelse av oppvekstmiljøet (EE) kunne bedre den regionspesifikke nevrale plastisiteten og kultiveringssuksessen av klekkerilaksen (**Artikkel II**). Etter syv ukers behandling viste EE-oppfostret parr høyere overlevelse etter utsetting i ferskvann enn kontrollindivider oppfostret i standard uniforme klekkeritanker. Den forbedrede kultiveringen ble imidlertid ikke gjenspeilet i signifikante endringer i telencephalons genuttrykk av plastisitetsmarkører.

Selv om strukturell EE har demonstrert å gi noen, dog inkonsekvente, fordelaktige effekter på kultivering i flere studier, er klekkeriledere motvillige til å implementere dette grunnet de hygieniske og operasjonelle begrensinger de kan føre med seg. Det er derfor viktig å utvikle alternative oppfostringsmetoder som kan bedre fiskens nevrale utvikling og samtidig være mer praktisk å innføre i produksjonen. Ett slikt alternativ er svømmetrening, noe som tidligere har blitt knyttet til økt overlevelse hos laksefisk etter utsetting. Ettersom løping er assosiert med økt nevroplastisitet i pattedyr, undersøkte vi i **Artikkel III** om svømming kunne virke som en alternativ strategi for å bedre laksens nevroplastisitet og kognisjon. Etter åtte uker med vedvarende svømming fant vi økt uttrykk av nevroplastisitet-relaterte gentranskripter i telencephalons transkriptom hos de trente laksene. Vi testet deres evne til romlig orientering i en labyrinttest, men fant ingen bevis for økt kognisjon hos den trente fisken. Til tross for at tidligere studier har rapportert positive fysiologiske effekter av svømmetrening, slik som økt vekst eller stressreduksjon, så er dette første gang at trenings-stimulert nevroplastisitet har blitt rapportert i laksefisk, noe som indikerer at svømmetrening bør utforskes som en potensiell måte å øke utsettingssuksessen av klekkerilaks.

Oppsummert bidrar resultatene i denne avhandlingen til en avansering av den anvendte fiskenevrobiologien i kultiveringssammenheng gjennom å karakterisere telencephalons nevroplastisitetsmarkører i laks på et mer detaljert nivå enn tidligere beskrevet. Vi viser at EE kan forbedre overlevelsen til parr i ferskvann, men at effektene av EE på nevroplastisitet er avmålte i de områder av telencephalon som ble studert her. Videre demonstrerer vi svømmetrening som et lovende nytt verktøy for forbedring av nevroplastisiteten i laks,

samtidig som vi understreker at trening har ytterligere fysiologiske fortrinn og er relativt lett å få implementert i klekkeriene. Vi foreslår derfor at fremtidige arbeid har som mål å validere den potensielle nytten av trening i optimaliseringen av klekkeribetingelsene for kultivering og at fremtidige undersøkelser søker å forstå sammenhengen mellom oppvekstmiljøet, hjernen og atferd.

Samenvatting

De Atlantische zalm (*Salmo salar*) is een iconische vissoort die oorspronkelijk in overvloed voorkwam, maar door menselijk handelen zijn de wereldwijde aantallen in de afgelopen decennia sterk gedaald, met name door overbevissing en verlies van leefgebied. Om afnemende wilde zalmpopulaties te ondersteunen worden jaarlijks miljoenen Atlantische zalmen gekweekt en vervolgens in rivieren vrijgelaten via uitzettingsprogramma's. De omstandigheden in de kwekerijen zijn echter zeer uniform en prikkelarm, wat tot gevolg heeft dat de vis zich naïef gedraagt na vrijlating in de natuur. Vaak vertonen gekweekte zalmen bijvoorbeeld minder efficïent foerageergedrag en vallen ze snel ten prooi aan predators, en mede daardoor hebben ze een lage overlevingskans in het wild. De effecten van het kweken op het gedrag van vissen zijn redelijk goed beschreven, maar de effecten op de hersenen – het orgaan dat omgevingsprikkels vertaalt in geschikt gedrag – zijn tot nu toe sterk onderbelicht gebleven. De weinige studies die de impact van de kwekerijomgeving op het centraal zenuwstelsel van vissen hebben bestudeerd, hebben met name de expressie van neuroplasticitiets- en neurogenesegenen in kaart gebracht in de gehele hersenen, of in grote hersenstructuren zoals de gehele voorhersenen (telencephalon). De hersenen zijn echter een zeer complex orgaan en bestaan uit een overvloed aan neurale subpopulaties, ieder met verschillende functionaliteiten en kenmerken die specifieke typen gedrag aansturen. Bij het kwantificeren van neuroplasticiteitsmarkers in de gehele hersenen bestudeert men een verzameling van al deze neurale populaties, en nuances tussen hersengebieden kunnen niet worden gedetecteerd. Het doel van dit proefschrift is om een beter inzicht te krijgen in de neurale verschillen in subregio's van het telencephalon tussen wilde zalm en gekweekte zalm, en hoe innovatieve kweekmethoden verbeteringen kunnen bewerkstelligen in de neurobiologie, het gedrag en de overleving van gekweekte zalm.

Allereerst hebben we een gedetailleerde karakterisatie gemaakt van de neurobiologie van juveniele wilde zalm en kweekzalm. Daarvoor hebben we de expressie van de neuroplasticiteitsmarker *bdnf* en de neurale activiteitsmarker *cfos* gekwantificeerd in vijf neurale subregio's van het telencephalon in wilde zalm en kweekzalm, zowel voor als na blootstelling aan een acute stressor (**Paper I**). We ontdekten dat de expressie van *bdnf* en *cfos* sterk varieerde tussen de bestudeerde subregio's, hetgeen bevestigt dat deze regio's ieder individuele eigenschappen hebben met betrekking tot hun reactie op externe stimuli. Vergeleken met wilde vis vertoonde de kweekvis van dezelfde genetische oorsprong na blootstelling aan stress een hogere neurale activering in het ventrale gebied van het

dorsolaterale pallium (Dlv), een hersengebied dat belangrijk is voor relationeel geheugen en ruimtelijke oriëntatie. Gestresste wilde zalm vertoonde een verhoogde expressie van *bndf* in het dorsomediale pallium (Dm), dat belangrijk is voor het emotioneel geheugen en de stressrespons, terwijl dit niet het geval was voor gekweekte zalm. Deze studie toont aan dat het bestuderen van neurale subregio's in het telencephalon bepaalde expressiepatronen kan onthullen die niet gedetecteerd kunnen worden wanneer het telencephalon in zijn geheel bestudeerd wordt. Verder hebben we aangetoond dat het kweken van invloed is op de neuroplasticiteit en neurale activatie in hersenregio's die belangrijk zijn voor leerprocessen en de stressrespons. Deze resultaten kunnen een mogelijke verklaring geven voor de gedragsverschillen die worden waargenomen tussen wilde zalm en gekweekte zalm.

Nadat we de neurale verschillen tussen wilde zalm en gekweekte zalm hadden gekarakteriseerd in de subregio's van het telencephalon, hebben we in **Paper II** onderzocht of de hersenplasticiteit en overlevingskansen van gekweekte zalm kunnen worden verbeterd door de kwekerij te verrijken met objecten als stenen en planten ('milieuverrijking'). Na zeven weken in een verrijkte omgeving hadden gekweekte zalmen een significant hogere overlevingskans na uitzetting in de rivier, vergeleken met een controlegroep die onder standaard omstandigheden was gekweekt. De verhoogde overlevingskans leek echter niet gepaard te gaan te met significante veranderingen in hersenplasticiteit.

Hoewel milieuverrijking een aantal (maar inconsistente) gunstige effecten heeft laten zien op het succes van uitzettingsprogramma's, zijn de eigenaren van kwekerijen terughoudend om milieuverrijking te implementeren vanwege hygiënische en operationele bezwaren. Het is daarom belangrijk om alternatieve kweekmethoden te ontwikkelen die een gunstig effect hebben op de neurale ontwikkeling van vissen, zonder praktische nadelen mee te brengen voor kwekerijen. Een van deze alternatieve kweekmethoden is het implementeren van zwemtraining, waarvan in eerdere studies al is gebleken dat het de overlevingskans van uitgezette zalm ten goede kan komen. Daarnaast is bekend dat rennen positieve effecten heeft op hersenplasticiteit in zoogdieren, maar de neurologische effecten van zwemtraining zijn nog nooit grondig onderzocht in vis. Daarom hebben we in **Paper III** onderzocht of zwemtraining de hersenplasticiteit en cognitie van Atlantische zalm kan bevorderen. Na acht weken zwemtraining vonden we verhoogde expressie van hersenplasticiteit-gerelateerde genen in het telencephalon van getrainde zalm. Echter, we hebben geen bewijs gevonden van verhoogde cognitie in getrainde vissen met betrekking tot het ruimtelijk geheugen bij het oplossen van een doolhof. Hoewel eerdere studies positieve fysiologische effecten van zwemtraining in vissen beschrijven, zoals een efficiëntere lichaamsgroei en verminderde

stress, is dit de eerste keer dat is aangetoond dat zwemtraining ook de hersenplasticiteit van zalmen kan bevorderen. Daarom lijkt zwemtraining een veelbelovende methode om de overlevingskansen van uitgezette zalm te bevorderen.

De resultaten die gepresenteerd worden in dit proefschrift leveren een bijdrage op het terrein van de toegepaste visneurobiologie doordat we de expressie van hersenplasticiteitsmarkers in het telencephalon van Atlantische zalm op een meer gedetailleerd niveau hebben gekarakteriseerd dan ooit tevoren. We laten zien dat milieuverrijking de overlevingskans van jonge zalm in de zoetwaterfase kan verbeteren, maar dat effecten van milieuverrijking op hersenplasticiteit beperkt zijn in de bestudeerde regio's van het telencephalon. We identificeren zwemtraining als een veelbelovende alternatieve kweekmethode om de hersenplasticiteit in zalm te bevorderen, en merken daarnaast op dat zwemtraining toegevoegde fysiologische voordelen biedt en relatief eenvoudig te implementeren is in de kwekerij. Daarom stellen we voor dat toekomstige studies zich richten op de validatie van zwemtraining als methode om kweekomstandigheden te optimaliseren, en dat verder onderzoek de samenhang tussen kweekomstandigheden, hersenen en gedrag nauwkeuriger in kaart brengt.

List of papers

Paper I

Neurobiology of wild and hatchery-reared Atlantic salmon: How nurture drives neuroplasticity

Daan Mes, Kristine von Krogh, Marnix Gorissen, Ian Mayer, Marco A. Vindas

Frontiers in Behavioral Neuroscience 2018, *in press*, doi: 10.3389/fnbeh.2018.00210

Paper II

Effects of environmental enrichment on forebrain neural plasticity and survival success of stocked Atlantic salmon

Daan Mes, Renske van Os, Marnix Gorissen, Lars O.E. Ebbesson, Ian Mayer, Marco A. Vindas

Submitted to Scientific Reports

Paper III

Brain training: Can swimming exercise enhance brain plasticity and cognition in Atlantic salmon?

Daan Mes, Arjan P. Palstra, Christiaan V. Henkel, Marco A. Vindas, Ian Mayer

Manuscript

Abbreviations

bdnf	***brain-derived neurotrophic factor***
BL	**body length**
bp	**base pair**
CAMK-II	**calcium/calmodulin-dependent protein kinase II**
cDNA	**complementary deoxyribonucleic acid**
CREB	**cyclic adenosine monophosphate response element binding**
Dl	**dorsolateral pallium**
Dld	**dorsal area of the dorsolateral pallium**
Dlv	**ventral area of the dorsolateral pallium**
Dm	**dorsomedial pallium**
Dmd	**dorsal area of the dorsomedial pallium**
Dmv	**ventral area of the dorsomedial pallium**
EE	**environmental enrichment**
F1	**first generation**
FL	**fork length**
GO	**gene ontology**
IGF	**insulin-like growth factor**
ISH	***in situ* hybridisation**
LTM	**long-term memory**
LTP	**long-term potentiation**
MAPK	**mitogen-activated protein kinase**
mRNA	**messenger ribonucleic acid**
NAcc	**nucleus accumbens**
neurod	***neurogenic differentiation factor 1***
pcna	***proliferating cell nuclear antigen***
PKC	**protein kinase C**
POA	**preoptic area**
qPCR	**quantitative real-time polymerase chain reaction**
RNA-seq	**ribonucleoacid sequencing**
$\mathbf{U_{opt}}$	**optimal swimming speed**
Vd	**dorsal nucleus of the ventral telencephalon**
Vv	**ventral nucleus of the ventral telencephalon**

1. Introduction

1.1 Salmon stocking

The Anthropocene, defined as the period of time during which human activities have had a significant impact on the Earth's climate and environment, is increasingly exerting pressures on the planet's natural resources and wildlife (Johnson et al., 2017). Anthropogenic effects, including habitat destruction, pollution and overexploitation, have led to the decline of many of the world's fish stocks, as well as a dramatic decline in fish biodiversity. For example, it has recently been reported that, in addition to six fish species having become extinct in the wild, 455 fish species are now listed as critically endangered (IUCN, 2016). Among a large number of management strategies to mitigate the impact of these anthropogenic effects, stocking is a commonly used management tool which entails rearing fish in hatcheries and subsequently releasing them into the wild, with the aim to augment the productivity of wild populations (Brown and Day, 2002; Araki and Schmid, 2010). There are many aims and reasons for stocking, and one of the most common aims is to enhance fish production for commercial and recreational fisheries (Bell et al., 2008). Importantly, fish stocking can also be used as a conservation tool (Cowx, 1994; Aprahamian et al., 2003) and in this context, stocking strategies may include:

1. Enhancement: stocking to supplement an existing stock where the production is less than the water body can sustain.
2. Mitigation: stocking conducted to mitigate lost production due to an activity that cannot be prevented or removed.
3. Restoration: stocking carried out after the removal or reduction of a factor which has been limiting or preventing natural production.

Thus, for various reasons, billions of fish, primarily salmonids, are stocked worldwide every year (e.g. Cowx, 1994; Nakashima and Sasaki, 2014; Klovach et al., 2015; Tompkins et al., 2015; Vercessi, 2015).

The Atlantic salmon (*Salmo salar*) is an iconic fish species, which historically has been of major cultural and economic importance throughout its geographical range. It has a complex and variable life cycle, where most individuals display an anadromous life history (**Fig. 1**), which means that they spawn in fresh water and migrate to sea during their lifetime (Thorstad et al., 2010). In general, anadromous adult Atlantic salmon spawn in freshwater between late autumn and early winter. Eggs hatch in spring and the newly hatched fish (alevins) typically spend several weeks in the gravel of the river bed while absorbing nutrients

from their yolk sacks, before emerging as fry. After spending 1-8 years in the river as parr (freshwater juveniles), salmon undergo smoltification: an adaptive specialization that involves morphological, physiological and behavioural changes to prepare them for migration to seawater and subsequent marine residence (McCormick et al., 1998). After their downstream migration, the post-smolts typically spend 1-5 years in sea before returning to their native streams to spawn (Thorstad et al., 2010). In contrast to Pacific salmon (genus *Oncorhynchus*), which are all semelparous, Atlantic salmon are iteroparous and can spawn multiple times during their lifetime. Because Atlantic salmon generally return to their native stream, every water body hosts a distinct salmon population with unique genetic and phenotypic adaptations, optimised for local conditions (García de Leániz et al., 2007).

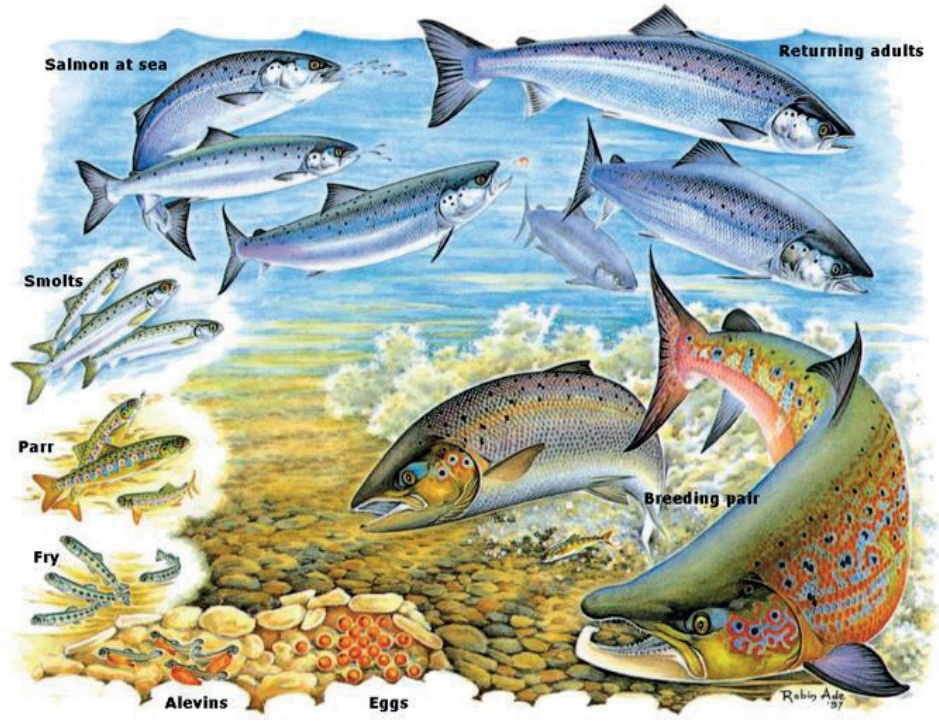

Figure 1. Generalised lifecycle of the Atlantic salmon. Illustration courtesy of the Atlantic Salmon Trust and Robin Ade.

The migratory lifestyle of Atlantic salmon exposes them to many anthropogenic threats, such as obstructed migration routes (Johnsen et al., 2010), pollution (Rosseland and Kroglund, 2010), over fishing and impacts from salmon farms, such as increased exposure to salmon

lice (reviewed by Thorstad et al., 2012). As a result, wild Atlantic salmon populations are declining throughout most of their geographical range (Parrish et al., 1998; Chaput, 2012). Even in Norway, historically home to some of the healthiest salmon stocks in the world, the number of wild salmon has more than halved in the past three decades (**Fig. 2**) and in a recent assessment of 148 Norwegian salmon populations, only 29 populations (20%) were assessed to be of good quality in terms of genetic integrity and population size (Thorstad et al., 2017; Thorstad and Forseth, 2017). Currently, it has been estimated that less than half a million wild adults return yearly to the ca. 465 salmon rivers in Norway (Jøranlid, 2017; Thorstad et al., 2017).

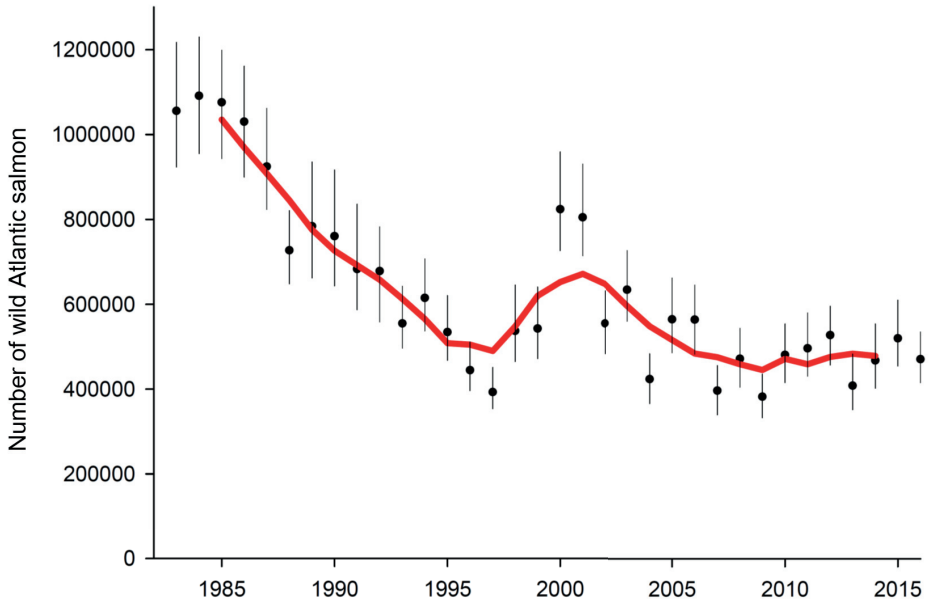

Figure 2. Estimated annual number of returning Atlantic salmon to the Norwegian coast in the period 1983-2016. Points show annual median values and vertical bars show the range between the minimum and maximum estimated number of salmon. The red line indicates the moving average based on five years. Modified from Thorstad and Forseth (2017).

Worldwide, tens of millions of *S. salar* are stocked in rivers every year (e.g. Finstad and Jonsson, 2001; Palmé et al., 2012; Maynard and Trial, 2013). In Norway alone, an estimated 8 million Atlantic salmon were released annually between 2005-2009 (Jøranlid, 2017). The majority of salmon stocking in Norway (approx. 4.5 million fish) involves restoration

stocking (Jøranlid, 2017). Restoration stocking is conducted after all fish have been eradicated in a river, for example by rotenone treatment (to combat the parasite *Gyrodactylus salaris*) or by liming (to counteract river acidification). Another large contribution to salmon stocking (approx. 2.2 million fish) comes from mandatory mitigation stocking by hydropower companies, who are obligated by Norwegian law to stock salmon to compensate for lost habitat, and restricted migration, due to hydro dam construction (Jøranlid, 2017). Additionally, more than one million salmon are stocked yearly by voluntary hatcheries, operated by local anglers or fishery owners.

Despite its historic use and current scale, fish stocking, and particularly mitigation and enhancement stocking, is a controversial practice. While the release of hatchery-reared fish can locally and temporarily increase total fish biomass, this is often at the expense of the existing wild population, as hatchery-reared fish compete with wild fish for limited natural resources (Amoroso et al., 2017). Further, stocking has been shown to reduce the genetic diversity and fitness of wild populations, which in the long term can accelerate their decline and extinction (Cross et al., 2007; Bartley and Bell, 2008). As the ultimate causes for population declines in managed fisheries are almost exclusively environmental pressures, often through anthropogenic effects, it is increasingly being argued that habitat restoration, rather than stocking, should be the main strategy for the conservation of wild diadromous fish populations (i.e. fish which migrate between the sea and fresh water; Araki et al., 2008; Araki and Schmid, 2010). However, habitat restoration is a time-consuming and expensive process. As an increasing number of wild salmonid populations are on the brink of extinction, stocking can in some cases be justified as an important management tool to ensure the survival of these threatened wild populations, while environmental pressures are alleviated. It is also important to acknowledge that, in the case of voluntary hatcheries, stocking activities provide a range of social and psychological benefits to the volunteers, which perpetuates stocking efforts, even if the beneficial impact on local stocks is contested by scientific arguments (Harrison et al., 2018). In summary, although stocking of juvenile fish is a controversial management tool, it remains a popular conservation measure among several stakeholder groups. When used properly, stocking can in some cases be a valuable and necessary tool to preserve vulnerable salmon populations, and will thus continue to be extensively used in the foreseeable future. However, this practice has to be used with care and many aspects of stocking programs require optimisation.

1.2 Adverse effects of hatchery-rearing

Careful planning, rigorous monitoring and sound hatchery practices are of paramount importance to create successful stocking programs. The work presented in this thesis focuses primarily on optimization of the rearing environment for juvenile fish in hatcheries. Although the rearing environment is an important factor in fish stocking practices, it is important to stress that many other factors need to be taken into consideration as well. For example, hatchery managers should conduct a careful assessment of the current wild population, define clear stocking objectives, select adequate broodstock fish and conduct crossing schemes which maximise genetic variation, ensure that the carrying capacity of the natural system is not exceeded, and determine the optimal timing for fish release (Cowx, 1994). Regarding the rearing environment, it is important that hatchery conditions are aimed at the production of fish that resemble their wild conspecifics as closely as possible in their genetic composition, morphology, physiology and behaviour, which together will ensure that stocked fish have a high post-release fitness. However, current hatchery technology resembles aquaculture practices, which maximises fish growth and production, instead of optimising fish quality from a stocking perspective (Brown, 2001). The average hatchery environment thus differs greatly from the wild environment (**Fig. 3**). Compared to natural conditions, hatchery-reared fish experience unnaturally high densities (Brockmark et al., 2010), high feeding rates at predictable times (Noble et al., 2007), relatively little physical exercise (Skilbrei and Holm, 1998; Hoffnagle et al., 2006), and a lack of variation in abiotic factors (Johnsson et al., 2014), physical structure (Salvanes et al., 2013), foraging opportunities on live prey (Sundström and Johnsson, 2001) and predator encounters (Salvanes, 2017).

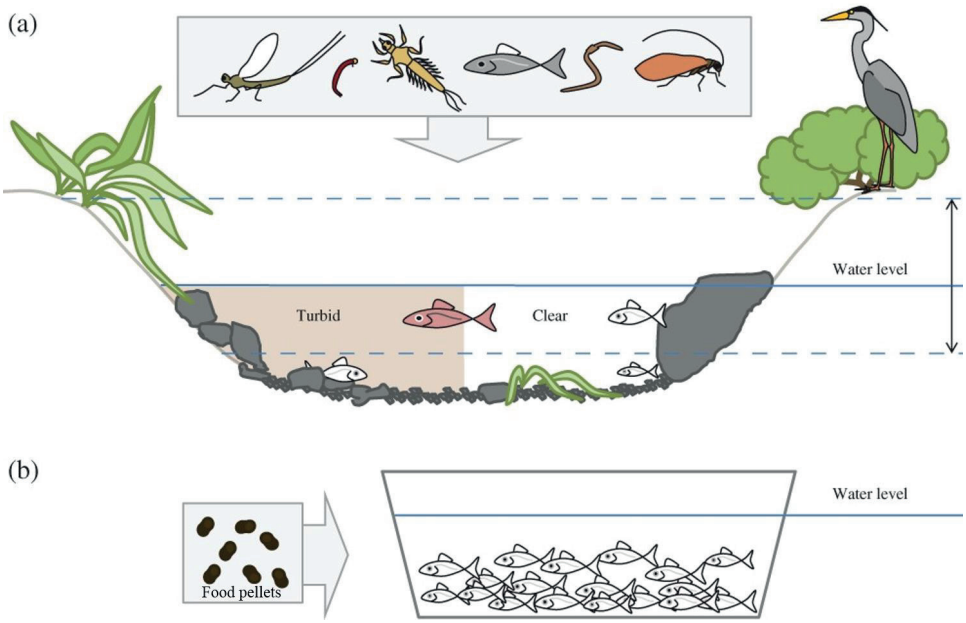

Figure 3. Some key environmental differences between a natural stream (a) and a conventional hatchery (b). Modified from Johnsson et al. (2014), with permission from John Wiley and Sons.

Behavioural responses are strongly shaped by earlier experiences (Brown, 2001; Brown et al., 2006; Ebbesson and Braithwaite, 2012; Vindas et al., 2018). Because hatchery-rearing conditions are impoverished compared to wild habitats, hatchery-reared fish are relatively naive and show deviating behavioural responses at time of release, compared to wild conspecifics (Johnsson et al., 2014). The homogeneous nature of hatchery environments, together with the high stocking density, high food abundance and lack of predation risk, favours individual fish which display a proactive (bold) rather than a reactive (shy) personality or coping style. That is, under intense hatchery conditions, proactive fish often outperform reactive fish, as their higher levels of aggression allow them access to more feed (Salvanes, 2017). In nature, however, a more proactive coping style will in some environments most likely lead to increased predator exposure (reviewed by Olla et al., 1998; Huntingford, 2004). In this context, selection of proactive individuals in the hatchery environment, together with a reported tendency for reduced antipredator behaviour displayed by hatchery-reared fish (Brown, 2001), make juvenile hatchery-reared salmon particularly prone to predation risks immediately after release into the wild (Henderson and Letcher,

2003). Furthermore, a lack of experience in handling live prey leads to impaired foraging behaviour (reviewed by Olla et al., 1998; Huntingford, 2004). That is, stocked salmon have a more selective dietary preference (Larsson et al., 2011) and show reduced stomach fullness compared to wild fish in the immediate weeks after release (Johnson et al., 1996), as well as higher ingestion rates of indigestible particles such as small rocks and plant material (Munakata et al., 2000). Because of these behavioural deficits, stocked fish show substantially lower survival rates than their wild counterparts (Jonsson et al., 1991; Johnson et al., 1996; Kallio-Nyberg et al., 2004; Jonsson and Jonsson, 2009; Thorstad et al., 2011) and it has been known for over half a century that the low quality of reared fish is directly related to their poor post-release performance (Burrows, 1969).

As outlined above, traditional hatchery-rearing can have many negative effects on fish development and behaviour, often resulting in low post-release survival of stocked fish. For this reason, future stocking practices should incorporate new strategies aimed at increasing the fitness of hatchery-reared fish. For example, efforts could be made to minimise the time spent in the hatchery, by releasing stocked fish at a young life stage, preferably as eggs. However, stocking of eggs or fry is not always possible, for instance when the carrying capacity of a habitat is (temporarily) greatly reduced in the case of a hydropower dam. Thus, stocking of parr and smolts is, in many cases, the only suitable management strategy. However, the current practice of releasing millions of hatchery-reared juvenile salmonids which are behaviourally naive, and consequently have a high post-release mortality risk, raises concerns from both a production (financial) and welfare (ethical) perspective. It is therefore imperative that we strive to optimise hatchery conditions to produce robust fish that display natural “wild type” behavioural responses, and thus have a higher survival rate following release into natural waters.

1.3 Innovative rearing methods

To mitigate the adverse effects of hatchery-rearing on fish behaviour, research efforts have been directed at enriching hatchery practices in order to mimic wild conditions more closely and thus produce fish which are better adapted for life in the wild (Näslund and Johnsson, 2014). A simple measure such as reducing fish density in hatcheries has been shown to mitigate crowding stress, reduce agonistic behaviour and improve growth rates (reviewed by Maynard et al., 1995; Johnsson et al., 2014). Consequently, several studies have reported higher post-release survival and adult return rates for salmonids reared at lower densities

(Martin and Wertheimer, 1989; Brockmark et al., 2010; Brockmark and Johnsson, 2010). However, the trade-off of rearing fish at lower densities is that the unit cost per smolt increases significantly. As a compromise, most hatcheries employ intermediate stocking densities for the production of fish used for stocking, while higher densities are used in traditional aquaculture production (Maynard et al., 1995).

An alternative production strategy to prepare salmon for life in the wild is to expose juveniles to "life skills training" during hatchery rearing. Juvenile salmon can be conditioned to avoid future contact with predators by exposing them to predator pheromones (e.g. Berejikian et al., 1999) or a plastic model of a predator such as a predatory bird (e.g. Roberts et al., 2014), preferably in combination with skin extract (alarm pheromones) of conspecifics, to condition fish to associate predator presence with danger (reviewed by Brown, 2001; Brown et al., 2011). Alternatively, naive fish may learn antipredator behaviour by social facilitation, meaning that fish may learn to avoid predators by observing a conspecific interacting with a predator (Brown, 2001). To improve foraging behaviour in hatchery-reared individuals, some studies have demonstrated that when fish are exposed to live prey in the hatchery, this earlier experience can improve future foraging rates on live prey (Sundström and Johnsson, 2001; Brown et al., 2003). Although a wealth of studies have shown that life skills training can improve the antipredator response and foraging behaviour of salmonids in the hatchery (e.g. Brown and Smith, 1998; Berejikian et al., 1999; Sundström and Johnsson, 2001), only a handful of studies have assessed the efficacy of these conditioning paradigms after release in the wild. Although some studies report positive effects of life-skills training on post-release survival (e.g. live prey conditioning; Czerniawski et al., 2011), several studies did not find increased survival after life-skills training (e.g. antipredator conditioning; Berejikian et al., 1999; Hawkins et al., 2007). Because the efficacy of life skills trainings thus appears to be limited, and because conditioning is a relatively labour intensive and thus a costly procedure, the effectiveness of conditioning training as a strategy to improve post-release survival of reared salmonids remains debated.

A much studied hatchery practice aimed at increasing fish quality and welfare is structural environmental enrichment (EE), which can be defined as "a deliberate increase in environmental complexity with the aim to reduce maladaptive and aberrant traits in fish reared in otherwise stimuli-deprived environments" (Näslund and Johnsson, 2014). For example, by adding physical structures (**Fig. 4**), the rearing environment shows a closer resemblance to wild conditions, which has implications for fish physiology and behaviour. For instance, in juvenile Atlantic salmon, EE has been shown to lower basal metabolic rate (Millidine et al.,

2006) and basal plasma cortisol levels (Näslund et al., 2013), suggesting that the presence of physical structures can reduce stress. A complex three-dimensional environment provides shelter opportunities from conspecifics, and EE can reduce conspecific aggression, as indicated by a lower amount of fin damage in fish reared in enriched tanks (Berejikian, 2005; Brockmark et al., 2007; Näslund et al., 2013). Structural EE also increases the propensity to seek shelter in novel environments (Roberts et al., 2011; Näslund et al., 2013). Such behaviour is likely to be beneficial to the salmon after release, as it may reduce exposure to predators. Atlantic salmon parr reared in EE also showed increased feeding rates on natural prey (Rodewald et al., 2011). Further, salmonids reared in EE conditions have an improved spatial learning capacity in maze tests, compared to fish reared in barren control tanks (Salvanes et al., 2013; Ahlbeck Bergendahl et al., 2016). Moreover, several studies have assessed the effects of EE on post-release survival in salmonids, and while some studies report increased survival in EE-reared fish (Maynard et al., 1995; Hyvärinen et al., 2013; Roberts et al., 2014), others find no effects (Brockmark et al., 2007; Fast et al., 2008; Tatara et al., 2009). These inconsistent results may be partly explained by the large variation in experimental variables between studies. Few studies on teleosts have used an experimental approach to determine the optimal conditions of EE, but there are indications that the duration of exposure to EE (Manuel et al., 2015; Ahlbeck Bergendahl et al., 2016), age of the fish (Manuel et al., 2015) and the type of enrichment (in mice; Lambert et al., 2005) can all affect the efficacy of EE in altering animal behaviour.

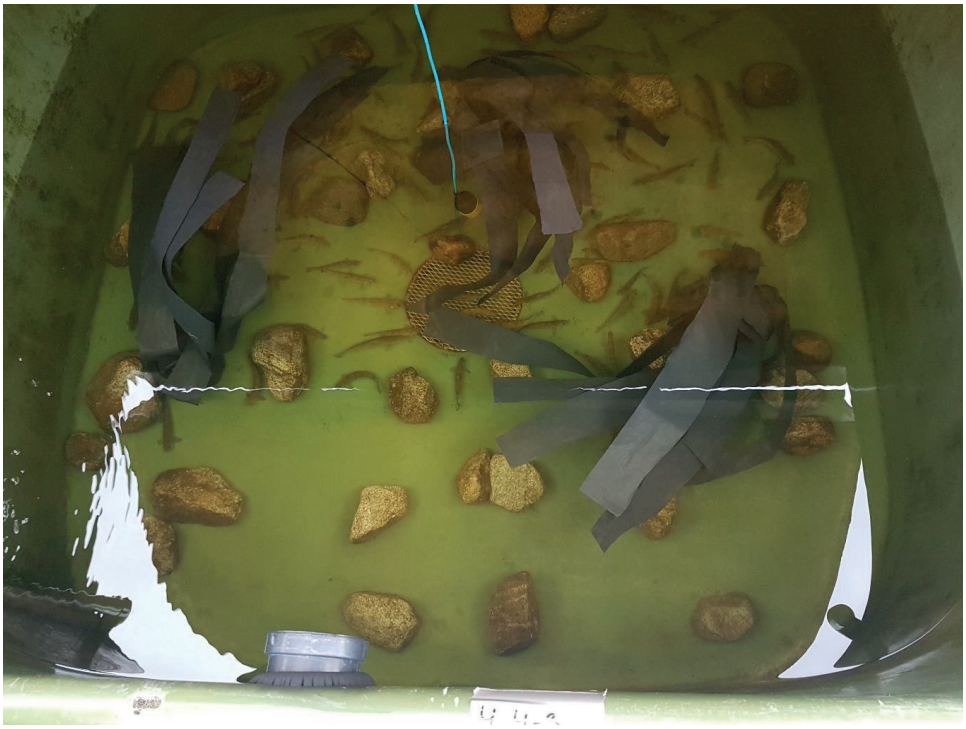

Figure 4. Example of structural environmental enrichment in an Atlantic salmon hatchery.

Environmental enrichment has thus been shown to have beneficial effects on the development of appropriate behavioural responses in hatchery-reared salmonids, and these improvements are, in some cases, translated into increased stocking success. However, hatchery-managers are reluctant to add structures to their tanks for practical and hygienic reasons, as structures obscure fish from view and cause waste accumulation, increasing the risk of reduced water quality and disease occurrence. Therefore, there is a strong need for the development of alternative hatchery strategies which can increase the behavioural fitness in reared fish without requiring the addition of structures to the rearing tanks or incurring increased labour demands. In this respect, swimming exercise seems a promising tool to investigate further. First, swimming exercise has already been shown to have many beneficial effects in fish rearing, including increased growth rates (reviewed by Davison and Herbert, 2013), improved feed conversion ratios (Palstra et al., 2015), and stress alleviation (reviewed by Huntingford and Kadri, 2013). Interestingly, mammalian literature has consistently shown that running exercise can improve cognitive performance, particularly in spatial orientation tasks such as maze tests (Vaynman et al., 2004; van Praag, 2008). In fish, the link between

swimming exercise and cognition has not been much investigated, aside from one study by Luchiari and Chacon (2013), who report that a 20-day exhaustive swimming regime improved the associative learning performance in a conditioning test in zebrafish (*Danio rerio*). In salmonids, inclusion of some form of exercise regime has improved post-release survival and adult return rates in some (Burrows, 1969; Cresswell and Williams, 1983; reviewed by Maynard et al., 1995), but not all studies (Lagasse et al., 1980; Evenson and Ewing, 1993; Skilbrei and Holm, 1998; Hoffnagle et al., 2006). Maynard et al. (1995) remarked that increased exercise-induced survival rates are only observed when fish have been exercised at moderate to high swimming speeds for a duration of at least 2 weeks. Other beneficial effects of exercise, such as increased growth rates, often occur when fish are exercised at their optimal metabolic swimming speed, i.e. the swimming speed at which the fish expends the least amount of energy per distance travelled (Davison and Herbert, 2013). It thus appears that the selection of an adequate swimming speed is an important parameter that drives the efficacy of exercise regimes in hatchery practices, both for optimising growth and for improving post-release survival in stocking programs. The mechanisms behind the positive effects of exercise on post-release survival in salmonids have traditionally been linked to increased swimming performance of exercised animals (Maynard et al., 1995). However, since recent mammalian studies have uncovered a link between physical exercise and increased cognition, this link should be further investigated in fish.

1.4 Fish neurobiology

The key organ which integrates stimuli from the environment and translates them into appropriate behavioural responses, is the brain. The fish brain is composed of several subdivisions which are shared by most vertebrates (Kotrschal et al., 1998), and a schematic representation of a teleost brain is depicted in **Figure 5**. At the caudal end of the brain, the spinal cord merges with the brain stem, which controls all somatosensory functions except for olfaction and vision, and is thus connected to nerves such as the facial nerves, lateral line nerves, etc. (Wullimann et al., 1996; Kotrschal et al., 1998). As in mammals, the brain stem houses the reticular formation, which controls basic maintenance and life support functions such as respiration, heartbeat frequency and wakefulness (Bernstein, 1970; Kotrschal et al., 1998). The hypothalamus and pituitary together play an important role in translating sensory inputs into appropriate neuroendocrine and behavioural responses. The hypothalamus is a major centre for integration of telencephalic information and is responsible for making the

decision to respond to both external and internal stimuli (Bernstein, 1970). The pituitary is attached to the base of the inferior lobe of the hypothalamus and is composed of the adenohypophysis and neurohypophysis (Kotrschal et al., 1998; Zohar et al., 2010). The neurohypophysis receives axonal projections from the hypothalamus, and the adenohypophysis contains hormone-secreting cells and is thus considered the glandular part of the pituitary, playing an important part in reproductive neuroendocrinology and the stress response (Wendelaar Bonga, 1997; Zohar et al., 2010). The cerebellum has traditionally been known to be involved in motor-control (Bernstein, 1970) and also plays a role in proprioception, eye movement and spatial orientation (Kotrschal et al., 1998). Notably, lesion studies have shown that fish with a damaged cerebellum show impaired performance in conditioning, emotional learning and spatial orientation tasks (Rodríguez et al., 2007), indicating that the cerebellum is also involved in higher-order processes such as perception and cognition. The optic tectum receives input from the afferent optic nerves and thus plays an important role in visual perception, as well as sensory processing and motor control via its efferent neuron projections (Kinoshita et al., 2006). The telencephalon is an important structure for emotional and relational learning, as well as decision making (Portavella et al., 2004; Broglio et al., 2005; Salas et al., 2006; Vargas et al., 2009). Finally, the olfactory bulbs, which are situated at the rostral end of the fish brain, contain olfactory receptors and are thus important for the perception of olfactory and gustatory stimuli (Bernstein, 1970).

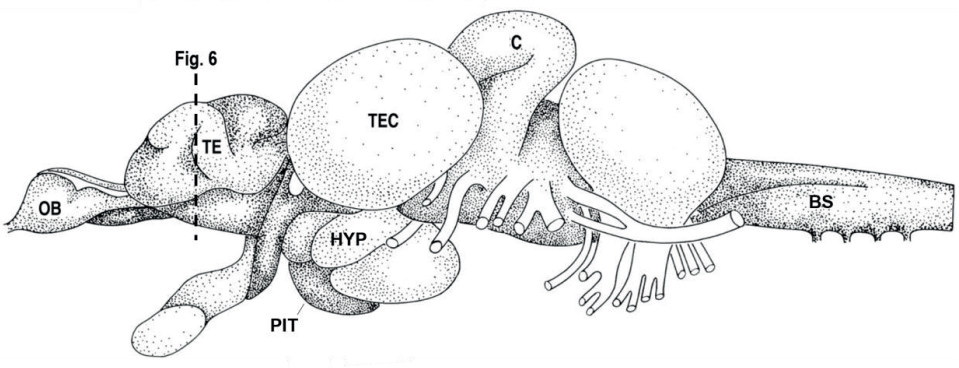

Figure 5. Lateral view of the goldfish brain. Abbreviations: brain stem (BS), cerebellum (C), hypothalamus (HYP), olfactory bulbs (OB), pituitary (PIT), telencephalon (TE), optic tectum (TEC). Indicated is the relative position of the transverse section depicted in **Fig. 6**. Modified from (Northcutt, 2006), with permission from John Wiley and Sons.

The fish brain shares numerous characteristics with that of other vertebrates, but is has classically been considered to be more primitive than the mammalian brain. However, the classical Aristotelian concept of *Scala naturae* (Hodos and Campbell, 1969), in which brain evolution has been described as a linear progression of complexity from "lower" (e.g. fish) to "higher" (e.g. mammals) animals, is being replaced by a new understanding of brain evolution, cognition and behaviour in vertebrates (Broglio et al., 2011). For example, even though the fish brain lacks a 6-layered pallium (i.e. mammalian neocortex, the brain region which in mammals is involved in higher-order brain functions related to sentience, such as sensory perception and cognition), fish still display so-called "higher functions" and cortical-like processes have been described extensively in fishes (Bshary and Brown, 2014). These higher functions in fish are mostly under telencephalic control. Within the fish telencephalon, there are several neuronal populations (**Fig. 6**) which are functionally equivalent to neural structures in the mammalian limbic system, which supports a variety of functions including emotion, motivation and memory (Morgane et al., 2005). For example, the fish's dorsolateral (Dl) pallium has been characterised as a functional equivalent to the mammalian hippocampus, which is involved in learning and spatial memory (Portavella et al., 2004; O'Connell and Hofmann, 2011). The dorsomedial (Dm) pallium is functionally equivalent to the mammalian amygdala, involved with decision-making and emotional reactions (Portavella et al., 2004; Vargas et al., 2009). Importantly, these proposed functional equivalences may in fact not be specific enough, since recent studies have suggested that the Dl and Dm are each composed of dorsal (Dld, Dmd) and ventral (Dlv, Dmv) neuronal subpopulations, each with distinct topology, connectivity patterns and, most likely, functionality (Broglio et al., 2015). In the subpallium, the ventral nucleus of the ventral telencephalon (Vv) has been proposed to be functionally equivalent to the mammalian lateral septum, which works in conjunction with both the hippocampus and amygdala to regulate decision-making and emotional learning (O'Connell and Hofmann, 2011; Singewald et al., 2011; Goodson and Kingsbury, 2013). Thus, the fish telencephalon plays an important role in cognition and decision making, and as such, it is a key neural structure that drives adaptive behaviour to environmental stimuli.

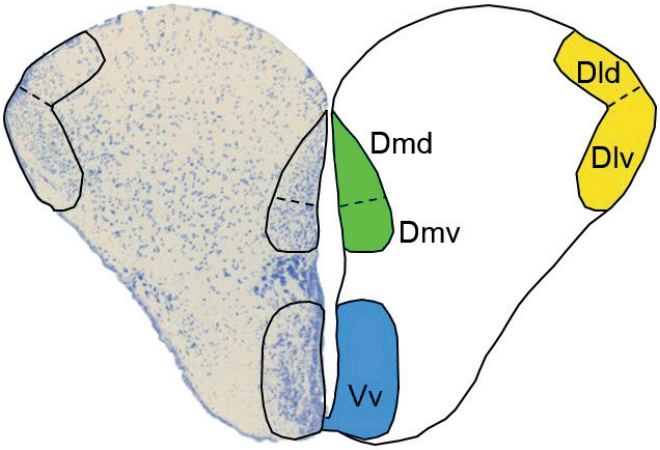

Figure 6. A transverse view of the Atlantic salmon telencephalon with a Toluidine Blue-stained left lobe and a schematic representation of the right lobe depicting the location of the dorsal (Dld) and ventral (Dlv) dorsolateral pallium, the dorsal (Dmd) and ventral (Dmv) dorsomedial pallium and the ventral nucleus of the ventral telencephalon (Vv).

Experiences in the rearing environment can alter fish behaviour by modifying the brain's organisation and structure (Ebbesson and Braithwaite, 2012). These modifications within the nervous system are known as 'neural plasticity', which is proposed to be driven by two major neural mechanisms: structural reorganisation of the neural circuits and biochemical switching of neural networks (Oliveira, 2009). Structural reorganisation encompasses the addition or removal of cells to the circuit (neurogenesis or apoptosis), as well as modification of the connectivity between neurons through axonal growth or modification of dendritic synaptic connections (Shors et al., 2012; Fischer, 2016). Biochemical switching mechanisms allow for the modulation of synaptic transmissions within existing fixed circuits through the use of neuroactive molecules (neuromodulators; Oliveira, 2009). Thus, by modulating circuit structure and connectivity, neural plasticity plays a pivotal role in the reinforcement of memory and perception and is critical for mounting appropriate behavioural responses to external stimuli (Shors et al., 2012). A plethora of molecules are associated with neural plasticity, and below we summarise several key genes and proteins which are routinely used as neurobiological markers for neural plasticity, with a particular focus on markers related to structural reorganisation.

Neurogenesis encompasses the formation of new neurons in the central nervous system. These newly born cells are incorporated into neural networks and may contribute to the

formation and establishment of new memories and learning processes (Clelland et al., 2009). For example, rodents subjected to voluntary running exercise show both increased cell proliferation rates in the hippocampus, as well as improved performance in spatial orientation tasks (van Praag, 2008; Voss et al., 2013). A commonly used marker to visualise cell proliferation is *proliferating cell nuclear antigen* (*pcna*). Pcna is a nuclear protein associated with DNA polymerase (Eisch and Mandyam, 2007). Pcna is therefore essential for DNA replication and Pcna protein levels peak during the S phase of the cell cycle (Eisch and Mandyam, 2007). Thus, by visualising Pcna protein levels or quantifying its mRNA abundance, it is possible to quantify the level of cell proliferation in the brain. It is important to note that Pcna occurs in all replicating cells and is therefore not a neurogenesis exclusive marker (i.e. Pcna presence may also indicate proliferation in cells other than neurons), but nonetheless, Pcna is much used as approximate marker for neurogenesis. Another way to visualise proliferating cells is to inject animals with bromodeoxyuridine (BrdU). BrdU is an analog of the nucleotide thymine and is thus incorporated into the DNA of replicating cells (Taupin, 2007). After sacrificing the animal, BrdU labelling can be retrieved through immunocytochemistry and all cells which have been replicating during the injection period can be visualised (Taupin, 2007). By increasing the duration of the injection period, this technique also allows assessment of neurogenesis on a longer temporal scale. Moreover, BrdU labelling can be used to study apoptosis: by comparing the number of BrdU-labelled cells between a subset of animals sampled one day post-injection, and a second subset of animals four weeks post-injection, van Praag et al. (1999b) were able to estimate cell survival rates. Some of the disadvantages of using BrdU are that it is a toxic and mutagenic substance and that the marker needs to be administered repeatedly during the experimental period, which may be stressful for the animals (Taupin, 2007).

In the adult mammalian brain, cell proliferation is limited to just two distinct zones: the subventricular zone of the lateral ventricles and the dentate gyrus, which is a substructure of the hippocampus (Barker et al., 2011). Fish, however, display neurogenesis in a multitude of proliferation zones throughout their entire lives (**Fig. 7**) and rates of cell proliferation in the teleost brain are one to two orders of magnitude higher than in the mammalian brain (Zupanc, 2006; Kaslin et al., 2008; Zupanc, 2008; Zupanc and Sîrbulescu, 2011). These higher cell proliferation rates, besides imparting remarkable neural plasticity, also contribute to the fact that upon neural damage, teleost fish species have an incredible capacity for regeneration of the central nervous system (Kaslin et al., 2008).

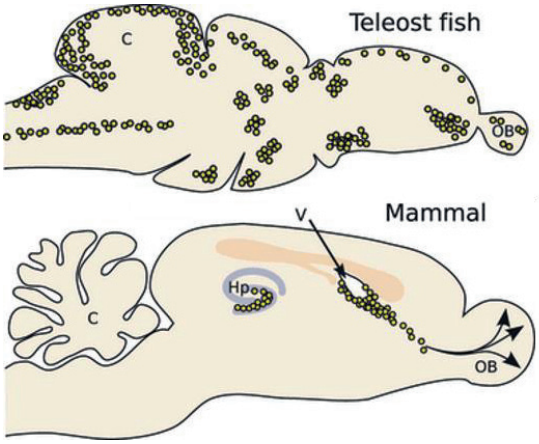

Figure 7. Sagittal view (cerebellum (C) to the left, olfactory bulb (OB), to the right) of generalised vertebrate brains, showing locations of proliferating cell populations that produce neurons in the adult brain (yellow dots). While cell proliferation is restricted to the lateral ventricles (V) and the hippocampus (Hp) in mammals, teleosts show proliferation in a large number of brain regions. Adapted from Barker et al. (2011) with permission from John Wiley and Sons.

Aside from neurogenesis and apoptosis, neural connectivity is another mechanism of structural reorganisation which plays a major role in learning, and is established through processes such as axon growth, as well as the growth or modification of dendritic spines (Ebbesson and Braithwaite, 2012). An extensively studied marker for neural plasticity in vertebrate research is *brain-derived neurotrophic factor* (*bdnf*). Bdnf is a protein from the neurotrophin family that promotes synaptic plasticity, as well as neurogenesis and cell survival, and is thus capable of altering the wiring of the brain in response to environmental cues (Mattson et al., 2004; Pang et al., 2004; Shors et al., 2012; Gray et al., 2013). Another marker for neural plasticity is the proneuronal gene *neurogenic differentiation factor 1* (*neurod*), which is important for neurogenesis (von Bohlen und Halbach, 2007) and dendritic spine stability (Gonda et al., 2009), and has been linked to improved cognitive performance and memory in fish (Salvanes et al., 2013).

Finally, a relevant group of neural markers to mention are the immediate early genes (IEG; Okuno, 2011). Immediate early genes, such as *cfos* and *activity-regulated cytoskeleton-associated protein* (*arc*), are not directly involved with structural neural plasticity, but they are highly expressed after neurons are activated. Therefore, by visualising IEG mRNA or protein levels in neural tissue, one can obtain a "snapshot" of neuronal activation patterns in

response to specific stimuli (Hoffman et al., 1993; Okuno, 2011; Pavlidis et al., 2015). Furthermore, IEGs may play an important role in neural processes such as consolidation of long-term synaptic plasticity and memory formation, as these cognitive processes are impaired in IEG-knockout mice (Okuno, 2011). Thus, visualisation of IEGs can provide an insight into neural circuit activation in response to certain environmental stimuli, and help us understand how these neural circuits may be linked to behaviour.

1.5 Neural plasticity and the hatchery environment

As outlined in **Sections 1.2** and **1.3**, the effects of hatchery-rearing on salmonid behavioural responses have been described to some extent. In contrast, the brain – the key organ that stores earlier experiences, processes environmental stimuli and subsequently generates appropriate behavioural responses – remains much understudied. However, there is some evidence of brain morphology differences between wild and domesticated salmonids. For example, hatchery-reared juvenile rainbow trout (*Oncorhynchus mykiss*) have been reported to have relatively smaller-sized brain structures such as the optic tectum, cerebellum, olfactory bulbs and telencephalon, compared to wild conspecifics (Marchetti and Nevitt, 2003; Kihslinger et al., 2006; Kihslinger and Nevitt, 2006). In addition, the same pattern was observed in hatchery-reared Chinook salmon (*O. tshawytscha*) alevins, which showed smaller total brain volumes compared to river-reared fish (Kihslinger and Nevitt, 2006). Although this may appear to be an important difference between hatchery-reared and wild fish, it is actually not possible to interpret how brain size relates to processing capacity (e.g. neurogenesis and brain plasticity differences) and, ultimately, behaviour and post-release survival (Ebbesson and Braithwaite, 2012; Johnsson et al., 2014). A few studies have compared the neurobiology of wild and hatchery salmonids in more detail and quantified expression levels of neural plasticity markers in the brain. For example, whole brain microarray analysis of male wild and hatchery-reared *S. salar* revealed 72 differentially expressed genes (Aubin-Horth et al., 2005). In the weakly electric fish *Brachyhypopomus gauderio*, BrdU labelling revealed greater rates of cell proliferation in the hindbrain, anterior midbrain and posterior midbrain in wild fish, compared to hatchery-reared conspecifics (Dunlap et al., 2011). Thus, the aforementioned studies have quantified cell proliferation markers on the level of whole brain or large brain structures. However, behavioural performance (e.g. spatial orientation or social memory) is under control of neural subregions

on a finer scale (e.g. Dl and Dm, respectively) and to our knowledge, no study to date has compared the neurobiology of wild and hatchery-reared salmonids on such a detailed scale.

In terms of effects of EE on brain structure, some studies have shown increased cerebellum size in fish reared under enriched conditions (e.g. Kihslinger and Nevitt, 2006; Näslund et al., 2012) but as mentioned above, it is difficult to infer functional implications of altered brain size. Interestingly, Salvanes et al. (2013) found that an 8-week enrichment period in Atlantic salmon parr increased mRNA expression of the neuroplasticity marker *neurod* in the whole telencephalon, and this was associated with increased cognitive performance in a spatial orientation task. Similarly, a study on zebrafish showed that one-week housing in enriched tanks increased cell proliferation in the whole telencephalon, evidenced by increased abundance of Pcna protein (von Krogh et al., 2010). In contrast, Manuel et al. (2015) reported decreased telencephalic *neurod* and *pcna* transcript abundance in zebrafish after six months of EE rearing. These contradicting results may be partly explained by differences in the enrichment protocol, but could also be confounded by the fact that all studies quantified gene expression in the telencephalon as a whole. As mentioned above, the telencephalon is comprised of a myriad of neuronal populations and networks with specific functionalities. In response to a stimulus, signalling molecules may be upregulated in one, but downregulated in another telencephalic region (Summers and Winberg, 2006). Importantly, when quantifying gene expression in the entire telencephalon, all regional expression patterns are pooled into one average expression level, which may not be reflected in the individual areas. With a wealth of knowledge regarding the effects of enrichment on the behaviour of fish, it is likely that EE should also lead to specific subregional changes in the telencephalon. For example, as EE can improve spatial memory in *S. salar* and *O. mykiss* (Salvanes et al., 2013; Ahlbeck Bergendahl et al., 2016) it could thus be expected that there would be specific EE-associated differences in neural plasticity in the Dl. However, to the best of our knowledge, no studies to date have assessed the effects of EE on the expression of neural markers within telencephalic neuronal populations on a detailed level.

Interestingly, a number of studies have previously investigated the effects of swimming exercise on post-release survival of salmonids (e.g. Burrows, 1969; Lagasse et al., 1980; Cresswell and Williams, 1983; Evenson and Ewing, 1993). In these studies, the authors focused mainly on the role of increased cardiovascular performance and exercise-enhanced growth on stocking success. Their work predated a wave of mammalian research that commenced in the late 1990s (e.g. van Praag et al., 1999b), demonstrating that physical running exercise promotes neurogenesis in the adult rodent brain, particularly in the dentate

gyrus, a substructure of the hippocampus, which is important for learning and memory (Vaynman et al., 2004; van Praag, 2008). Since then, a vast body of literature has described the effects of exercise on mammalian neurogenesis, mediated through growth factors, neurotransmitters and neurotrophic factors, and how these neural effects can boost cognition and even prevent cognitive decline (reviewed by Ma et al., 2017). Even though the mammalian and teleostean ancestral lines diverged around 400 million years ago, mammals and fish share many conserved neurochemical, topological and functional neural characteristics (Winberg and Nilsson, 1993; O'Connell and Hofmann, 2011; Broglio et al., 2015). Furthermore, cell proliferation is more widespread in the adult fish brain compared to the mammalian brain (Barker et al., 2011; Zupanc and Sîrbulescu, 2011). Therefore, it has been hypothesised that exercise-induced stimulation of neurogenesis and cognition should also occur in fish (Huntingford and Kadri, 2013). However, to date, the link between swimming exercise and neurogenesis/neural plasticity in fish has received remarkably little attention. Interestingly, in a recent study, Fiaz et al. (2014) subjected zebrafish larvae to a six-day intermittent swimming exercise regime and found increased expression of markers which have been associated with cell differentiation and cell growth in the mammalian hippocampus (Ma et al., 2009; Chung et al., 2011). Thus these preliminary findings hint towards the possibility that exercise-induced neurogenesis might also occur in the fish brain. However, further research is needed to confirm this hypothesis and explore whether swimming exercise may also promote neural plasticity in fish, and whether this may be linked to increased cognition.

1.6 Knowledge gaps

In summary, previous studies have established that rearing fish under uniform, stimulus-poor hatchery conditions has negative effects on fish behaviour and stocking success and that these negative effects may be partly alleviated by enhancing the rearing environment. Despite the central role of the brain in driving fish behaviour and, consequently, post-release survival, little is known about the effects of the rearing environment on fish neurobiology. A few studies have compared neural plasticity and neurogenesis markers between wild vs. hatchery-reared fish or between fish reared in conventional vs. enriched environments, but these comparisons have often been made at either the level of the whole brain or at the level of large brain structures (e.g. the entire telencephalon, midbrain, hindbrain etc.). However, recent developments in fish neurobiology provide new insights into the complexity of the fish brain.

These studies have provided detailed maps of neural topography and functionality (Folgueira et al., 2004a; Northcutt, 2006; Salas et al., 2006), which can be used to conduct more region-specific studies on the control of behavioural responses by specific neural networks. In this context, it is important to bear in mind that studies on large brain structures provide an oversimplified image of the link between brain and behaviour at best, and there is an increased need for a better understanding on how the environment affects the fish brain, and consequently, fish behaviour. Additionally, current methods for hatchery optimisation in stocking programs encompass strategies that are labour intensive (e.g. antipredator or foraging conditioning) or that raise practical concerns for daily hatchery management practices (e.g. environmental enrichment). Thus, there is need for the identification of novel, practical hatchery techniques that can enhance fitness traits such as behaviour and cognition, without interfering with hatchery operations.

2. Aims of the study

The general objective of this thesis is to investigate how improvements to the hatchery environment may increase the fitness of hatchery-reared Atlantic salmon in stocking programs. This was done by assessing the effects of hatchery rearing conditions on neural plasticity, as well as behavioural responses which are of critical importance for post-release survival. To achieve this objective, three sub aims were identified:

1. Identify the specific telencephalic subregions in which neuroplasticity markers are differently expressed between juvenile wild vs. hatchery-reared Atlantic salmon (**Paper I**).

2. Determine how environmental enrichment may affect neuroplasticity and post-release survival of hatchery-reared Atlantic salmon parr (**Paper II**).

3. Assess the potential benefits of sustained swimming exercise as a novel hatchery strategy to increase neural plasticity and cognitive performance of hatchery-reared Atlantic salmon (**Paper III**).

3. Methodological considerations

3.1 Fish origin

All experiments presented in this thesis were conducted using Atlantic salmon parr from the river Imsa population. The river Imsa is a 1-km long river located close to the city of Stavanger in south-western Norway (58°50'N; 5°58'E). The river carries water from Lake Liavatn (29 meters above sea level) downstream towards the ocean, into the Høgsfjord (**Fig. 8**). A 2-m high barrier at the upstream limit of the river prevents fish from migrating upstream into Lake Liavatn. The annual mean water discharge of the river Imsa is 5.1 m^3 s^{-1}, varying from 10 m^3 s^{-1} in autumn and winter to 2 m^3 s^{-1} in summer (Jonsson et al., 1988; Bergesen et al., 2017), and the water temperature fluctuates between 2°C in winter to 20°C in the summer (Jonsson et al., 1998).

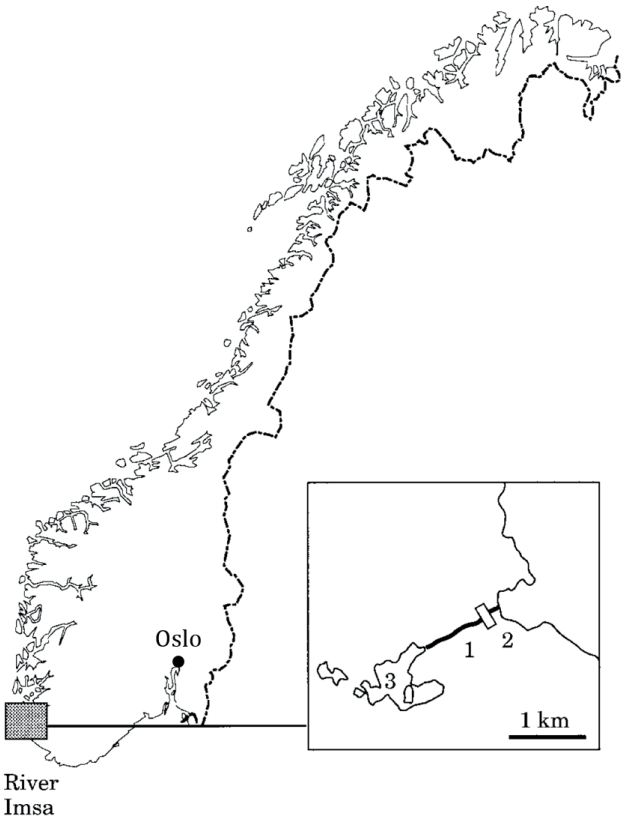

Figure 8. The location of the river Imsa in south-western (1), the fish trap (2) and Lake Liavatn (3). Modified from Jonsson et al. (1998) with permission from Wiley and Sons.

In 1975, a Wolf-type fish trap was constructed at the mouth of the river, approximately 150 m upstream from the estuary (**Fig. 8**). This fish trap intercepts all upstream and downstream migrating fish and because of continuous fish monitoring efforts since the 1970s, the populations of Atlantic salmon, brown trout (*Salmo trutta*) and European eel (*Anguilla anguilla*) in the river Imsa are among the best studied in Norway. In 1978, the Norwegian government opened a research station ("Forsnkingsstasjon for ferskvannfisk") near the mouth of the river Imsa, and in 1988 this research station was acquired by the Norwegian Institute for Nature Research (NINA), which has, since then, been extensively involved with fish monitoring and stocking in this river (Bergesen et al., 2017). The salmon population in the river Imsa is currently at a historic low, and in 2016, aside from 106 adults of stocked origin, only 15 wild adults returned to the river to spawn (Bergesen et al., 2017). The majority (78%) of wild Imsa Atlantic salmon smoltify at two years of age, while 14% and 7% smoltify at one and three years of age, respectively (Jonsson et al., 1998). Downstream smolt migration typically occurs in mid-May and migration timing is water temperature-dependent (Jonsson and Ruud-Hansen, 1985). Since the 1980s, the research station and NINA hatchery have been rearing thousands of first generation (F1) offspring from wild parents every year, as part of their stocking efforts. Offspring are reared in river water from the Imsa. Because all migrating fish are intercepted and both wild fish as well as F1 hatchery-reared offspring from wild parents are available, the river Imsa is a perfect system to perform comparative studies of wild and hatchery-reared fish, as well as to study the effects and efficacy of stocking.

3.2 Experimental setup

In **Paper I**, we compared the expression of neuroplasticity markers in telencephalic subregions between wild and first generation hatchery-reared fish from the Imsa strain, under basal and acute-stress conditions. Fish were subjected to confinement stress, which is a commonly used paradigm which exposes fish to a standardised stressor (e.g. Pottinger et al., 1992), which in our case entailed a 30-min isolated confinement in a 10-L bucket filled with 2 L of river water. Working with wild salmon poses a number of challenges, such as the limited availability of wild fish and the impact of the capture procedure on their homeostasis and specifically, their stress response. Our initial approach was to sample wild fish which were intercepted in the Wolf trap during downstream migration, as we hoped that this capture method would be less stressful to the fish than capture by net directly from the river. However, after a pilot study in which we compared the endocrine stress response (basal vs. acute stress

response) between hatchery fish and wild fish sampled from the Wolf trap (**Fig. 9**), it was determined that this method was not appropriate. In short, we found that smolts sampled from the Wolf trap displayed very high levels of plasma cortisol at basal conditions, indicating that this capture method had been stressful to the fish. Therefore, in order to minimise capture-related stress in wild fish, we decided to capture wild fish by electrofishing instead. In electrofishing, an electric current is passed through the water, which stuns the fish for a few seconds, while invoking involuntarily movement towards the anode (Bohlin et al., 1989). Adopting this methodology proved more successful than the pilot study, with wild salmon parr captured by electrofishing displaying a normal endocrine stress response (i.e. elevated plasma cortisol levels) when subjected to an acute confinement stress (**Paper I**). To control for effects of electrofishing, hatchery-reared fish were also shocked for 2 seconds prior to sampling.

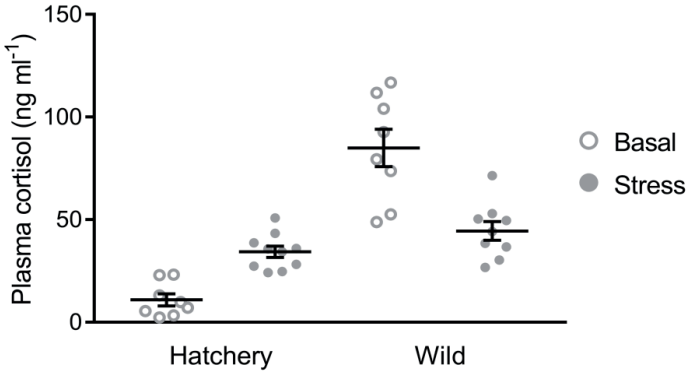

Figure 9. Pilot study showing that plasma cortisol levels at basal and post-acute stress conditions were elevated in wild Atlantic salmon intercepted by the Wolf-type fish trap. Black bars represent mean ± SEM values and grey circles show individual values.

Our aim in **Paper II** was to assess the effects of EE on neural plasticity and post-release survival in Atlantic salmon parr. Many different EE paradigms can been used, and experimental setups often differ in parameters such as the type of objects used, rearing density and duration of the exposure. For our study, we decided to replicate the experimental setup (**Fig. 10**) as described by Salvanes et al. (2013), as they report that their particular experimental setup increased neuroplasticity (*neurod* mRNA abundance in the whole telencephalon) and cognition (increased performance in a spatial orientation task) in Atlantic

salmon which were of similar age and from a comparable origin as our fish (i.e. F1 offspring from wild parents from a Norwegian river).

Figure 10. The experimental setup for **Paper II**, depicting a control (left) and enriched (right) tank.

In **Paper III**, fish were subjected to sustained swimming exercise for eight weeks, after which their neural plasticity and cognitive capacity in a spatial orientation task were assessed. To our knowledge, there are no published studies that have assessed the effects of swimming exercise on brain plasticity and cognition in salmonids. Therefore, we had to refer to mammalian work and salmonid swimming physiology studies to predict appropriate experimental conditions for this study. First, we had to select an appropriate swimming speed. The known beneficial effects of exercise in fish, such as improved growth rates and feed conversion efficiency (reviewed by Davison and Herbert, 2013), usually occur near optimal swimming speed (U_{opt}), which is the swimming speed at which the fish spends the minimum amount of energy to cover a certain distance (Tudorache et al., 2013). At swimming speeds below U_{opt}, fish may allocate excess energy towards negative behaviours such as aggression (type II allostatic overload; McEwen and Wingfield, 2003), while swimming speeds above U_{opt} may lead to stress and anaerobic respiration, which consequently produces an oxygen debt, leading to chronic fatigue (type I allostatic overload; McEwen and Wingfield, 2003). Interestingly, Maynard et al. (1995) observed that earlier studies, which determined the effects of swimming exercise on salmonid post-release survival in stocking programs, only found positive effects of exercise on post-release survival when animals were exercised at high swimming speeds, suggesting that the beneficial effects of exercise on stocking efficiency are

also speed-dependent. The optimal swimming speed varies with fish size (e.g. Palstra et al., 2015), and from pilot studies we know that U_{opt} for 200-mm Atlantic salmon parr is 3.8 body lengths (BL) s^{-1} (A.P. Palstra, unpublished data). The maximum swimming speed of Atlantic salmon parr from the Imsa population is known to be between 5.5 – 5.8 BL s^{-1} for individuals of 140 mm fork length (FL), which is close to the size of fish used in our experiment (123 mm FL). Thus, we had a good indication that a swimming speed close to 3.8 BL s^{-1} (47 cm s^{-1} for salmon of 123 mm FL) would represent an exercise regime that would lead to positive physiological effects, and still be well within the aerobic scope of the animals. As this study was conducted with a future application in fish stocking practices in mind, we verified that a flow rate near 47 cm s^{-1} is also ecologically relevant: preferred water velocities of wild Atlantic salmon parr in their natural habitat have been reported to be between 20-60 cm s^{-1} (reviewed by Symons and Heland, 1978; Heggenes et al., 1999; Armstrong et al., 2003). In order to ensure that smaller individuals would also be able to sustain the exercise regime, we selected an exercise speed of 43 cm s^{-1} (3.5 BL s^{-1} for the average-sized fish).

Salmon were exercised in a 3,600-L Brett-type swim flume (Brett, 1964), which is suitable for forcing groups of fish to swim at a predetermined speed (e.g. Palstra et al., 2015; Mes et al., 2016). In this flume, two groups of fish are housed in adjacent 525-L compartments, where fish in the outer compartment are subjected to a specific flow rate (exercised fish), while this flow is diverted from the fish housed in the inner compartment, which thus serve as unexercised controls (**Fig. 11**). While this swimming flume is an effective experimental setup to study the effects of swimming exercise on fish physiology and behaviour, it is not suitable for rearing large numbers of fish. Most salmonid hatcheries use circular rearing tanks for fish production, and to ensure that our results would be readily applicable to hatchery practices, we also included a swimming treatment in a standard hatchery tank. In this tank, we were able to achieve a maximum flow rate of 36 cm s^{-1} (2.9 BL s^{-1}) near the tank wall at the water surface, because the maximum flow rate was limited by the diameter of the outflow pipe. This flow rate is slightly lower than that of the Brett-type flume, but as swimming in circular trajectory incurs slightly higher energetic costs than swimming in a straight line (Domenici et al., 2000), this difference is likely to be negligible. Inside a circular tank, flow rates will inevitably be higher near the tank wall compared to the centre, and flow rates generally decrease with depth. In our experimental tank, measured surface flow rates varied from 10 cm s^{-1} (0.8 BL s^{-1}) in the centre to 36 cm s^{-1} (2.9 BL s^{-1}) along the wall, while flow rates near the bottom of the experimental tank varied from 5 cm^{-1} (0.4 BL s^{-1}) at the centre to 27 cm^{-1} (2.2 BL s^{-1}) along the wall. Thus, by manoeuvring themselves within the tank, fish could

'choose' their preferred swimming speed. For control treatment, a group of fish was housed in an identical tank with negligible (< 5 cm s^{-1}) water flow. An additional advantage of including a volitional swimming speed treatment is that animals often prefer to swim near U_{opt} (Tudorache et al., 2011), and a tank with a variable water flow thus allows fish of all sizes to find the right flow rate that subjects them to their individual U_{opt}. Furthermore, we hypothesised that there may be positive welfare effects associated with voluntary exercise, as fish can decide to reduce their metabolic expenditure which may have stress-alleviating effects. In summary, this experimental setup allowed us to compare the effects of different exercise regimes, i.e. a "forced" swimming speed (in the Brett-type flume) and a 'volitional' swimming speed (in standard hatchery tanks) on telencephalic neural plasticity.

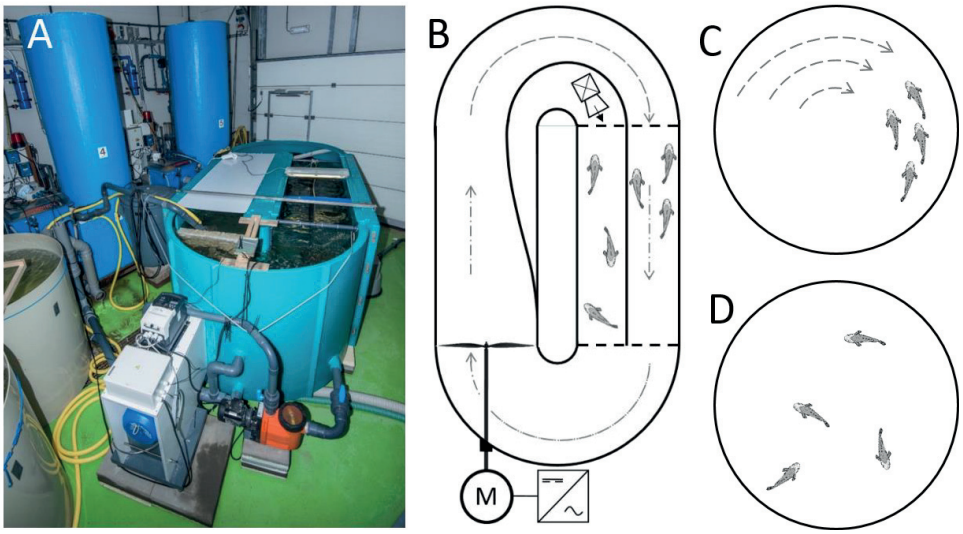

Figure 11. The experimental setup in **Paper III**. A side view (**A**; photo by Yoeri van Es) and schematic top view (**B**) of the 3,600 L swim-flume that was used to force fish to swim at a sustained speed of 3.5 BL s^{-1}. An electric motor (M) propelled an impeller within the flume, which created a water current which was directed through the outer compartment, forcing fish to swim at a predetermined speed, while fish in the inside compartment were not subjected to an increased water flow and thus served as unexercised control animals. Fish exercised at a volitional swimming speed were housed in cylindrical tanks with high water flow (**C**), while their respective controls were housed in identical tanks, without additional flow (**D**).

To assess the effects of exercise on cognition, fish were subjected to a maze test after three and eight weeks of swimming. We used the four-armed maze setup (**Fig. 12**) and procedures as described by Salvanes et al. (2013), because they showed robust treatment effects (EE-rearing vs. control rearing) on spatial memory performance of juvenile F1 offspring of wild Atlantic salmon. Due to logistic constraints, we were not able to test both forced and volitional groups in the maze test and thus we had to prioritise one of these groups (and their respective controls). While in mammals, both forced and volitional exercise regimes have been reported to enhance spatial learning (Fordyce and Farrar, 1991; Fordyce and Wehner, 1993; van Praag et al., 1999a; van der Borght et al., 2007), it seems that voluntary exercise is more effective at inducing hippocampal neurogenesis than forced exercise, as evidenced by the large volume of mammalian studies which have implemented voluntary exercise regimes (reviewed by van Praag, 2008). Therefore, we subjected fish from the volitional exercise regime, and their respective controls, to the maze test at three and eight weeks after the onset of swimming.

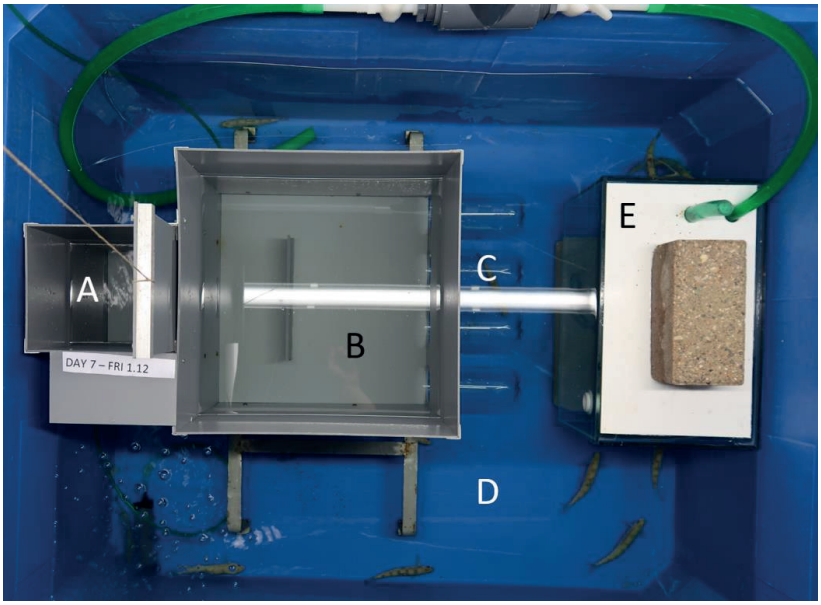

Figure 12. Experimental setup of the maze test used in **Paper III**. Fish were placed in a start box (**A**) and after a hatch was remotely opened, fish could explore the arena (**B**), which was connected to one open transparent arm (**C**) and three adjacent closed arms. After exiting the open arm, fish entered the holding tank (**D**), where they remained until all fish were tested. Three conspecifics housed in an aquarium adjacent to the arms (**E**) served as social stimulus.

3.3 Neuroplasticity

3.3.1 *In situ* hybridisation and cell quantification

In situ hybridisation (ISH) is a histological technique which enables visualisation of specific DNA or mRNA fragments within tissue (Jin and Lloyd, 1997). In this thesis, we used digoxigenin-labelled complementary RNA probes ('riboprobes') to visualise *cfos* and *bdnf*-expressing cells in parallel transverse sections of telencephalon tissue. The ISH protocol was conducted as described by Ebbesson et al. (2011), and slides from all treatments were stained simultaneously in the same Coplin jar to avoid differences in colouration due to handling effects. After staining, all slides were photographed and transcript-expressing cells were counted in the Dld, Dlv, Dmd, Dmv and Vv subregions by a semi-automated approach using the Fiji platform (Schindelin et al., 2012) in ImageJ2 (Rueden et al., 2017). To this end, images were converted into grayscale (8 bit), the neural area of interest was manually selected (**Fig. 13C**) and the black and white threshold was adjusted to match the labelled cells in the original image (**Fig. 13D, E**). Then, all labelled cells that measured between 15-500 pixels were automatically counted using the 'Analyse Particles' command (**Fig. 13F**). This quantification process is binary and cells are either counted as transcript-expressing or non-expressing, based on the labelling intensity of the digoxigenin riboprobes. Because the number of available brain sections differed per fish, we corrected for the number of counted sections by calculating the average number of labelled cells per section of each subregion.

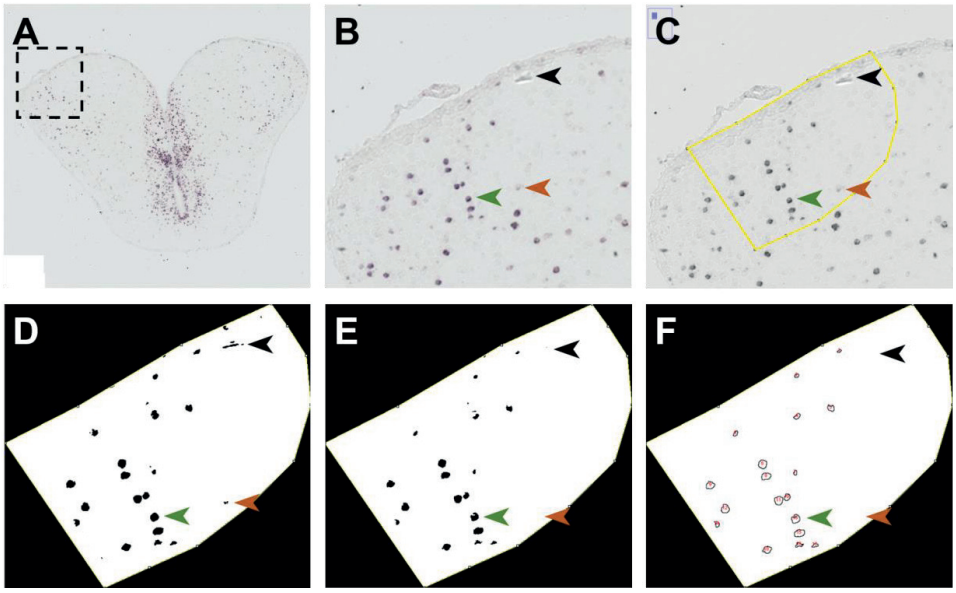

Figure 13. Semi-automated quantification of transcript-labelled cells. Panel **A** depicts the entire telencephalic section and the outlined region (dashed line) is depicted in panel **B**. To quantify the number of labelled cells, the area of interest was manually selected (**C**), the image converted to greyscale (**D**), the black and white threshold was adjusted to resemble the original picture as closely as possible (**E**), and only cells between 15-500 pixels were automatically quantified by the software (**F**). The green arrow indicates a cell which shows sufficient labelling to be quantified in panel **F**, while the orange arrow indicates a cell with insufficient labelling intensity to be quantified. The black arrow shows material which is not a labelled cell, and which is effectively excluded from the quantification analysis by adjusting the black and white threshold in panel **E**.

Many alternative methods exist to quantify the number of cells of interest in histological sections. For example, the number of counted cells can be expressed relative to the surface area or volume of the brain region of interest (e.g. von Krogh et al., 2010; Sørensen et al., 2011), or alternatively, the total number of labelled cells in an entire brain area can be estimated by multiplying the number of labelled cells per section by the distance between the serial sections (e.g. van Praag et al., 1999b). Both of these methods incorporate a size-correction factor, which can be useful when aiming to correct for unwanted size differences between treatment groups. In **Paper I**, we compared the region-specific telencephalic expression of *cfos* and *bdnf* between wild and hatchery-reared individuals. The aim of

stocking programs is to produce fish which resemble their wild conspecifics as closely as possible, but due to favourable rearing conditions, such as a lipid-rich diet, hatchery-reared individuals are generally significantly larger compared to their wild age-matched counterparts (Jonsson and Jonsson, 2009). Thus, unequal body size is an inherent and unavoidable difference between the two experimental groups, and the implementation of size corrections in the quantification of labelled cells could thus have disproportionate corrective effects when comparing the two groups. For this reason, we chose to report the average number of stained cells per telencephalic section, without correcting for area size, to provide an objective characterization of the expression patterns of these neuroplasticity markers.

3.3.2 Microdissections and qPCR

Quantitative real-time polymerase chain reaction (qPCR) is a methodology which allows for quantification of target DNA molecules (Bustin, 2002), and can be used to quantify expression of specific mRNA fragments extracted from a tissue of interest. To this end, total RNA is extracted from biological tissue and after a DNase treatment, to ensure all genomic DNA is removed, cDNA is subsequently synthesised from the RNA isolate by reverse transcription (Bustin et al., 2009). Primer pairs should be designed to specifically target the gene of interest, and preferably span exon-exon junctions to avoid the risk of false positive results from genomic DNA contamination (Bustin, 2000). In qPCR, these gene-specific primers are used to amplify target cDNA in the tissue of interest through consecutive cycles (generally approximately 40 cycles) of polymerase chain reaction (PCR), where the synthesised DNA is labelled by a fluorescent signal. The fluorescence of each sample is measured after each replication cycle and this measure of fluorescence can be translated into a quantitative gene expression value by comparing the signal to a standard curve (Bustin, 2000). Gene expression values are expressed relative to the expression of reference genes, allowing comparison of relative expression levels between samples (Vandesompele et al., 2002). In this process, it is important to first verify the stability of the reference genes between samples (e.g. Vandesompele et al., 2002; Andersen et al., 2004; Pfaffl et al., 2004; Silver et al., 2006).

Several previous studies have used qPCR to assess the effects of EE on the expression of neuroplasticity markers in the entire telencephalon (e.g. Salvanes et al., 2013; Manuel et al., 2015), but to date, no study has used qPCR to study the effects of EE of neuroplasticity marker expression within specific telencephalic subregions. Using microdissections, it is possible to isolate tissue from distinct neural populations (e.g. Wood et al., 2011; Basic et al., 2013;

Vindas et al., 2017), which can subsequently be used for qPCR analysis. In **Paper II**, we used a modified 23 gauge needle to microdissect regions of interest from frozen 100 µm serial telencephalon sections.

3.3.3 RNA sequencing

RNA sequencing (RNA-seq) is a technique that can detect and quantify transcript abundance of thousands of genes simultaneously in tissue samples. The field of RNA-seq is advancing rapidly and many sequencing technologies currently exist, including Roche/454, Illumina/Solexa, Life/APG and Nanopore sequencing (Metzker, 2009). In **Paper III**, we used Illumina technology to map the transcriptome of exercised and non-exercised Atlantic salmon parr. Specifically, we used the Illumina HiSeq2500 system to sequence millions of 50 base pair (bp) single-end reads from the transcriptome per sample. These short reads were then annotated to the Atlantic salmon reference genome (Lien et al., 2016). After correcting for gene length, the relative number of reads can be expressed for each individual fish, and differences in expression patterns can be identified between treatment groups (Robinson et al., 2010). Additional gene ontology (GO) analysis was used to group transcripts into categories by common biological properties, thus providing greater insight into the functional relevance of observed differences in expression patterns (Young et al., 2010). To perform the GO analysis, we employed the R package GOseq, including correction for transcription length (Young et al., 2010).

3.3.4 Neuroplasticity – methodological considerations

As outlined in the previous sections, we have used three different methodologies to study the expression of neuroplasticity markers, all of which visualise or quantify mRNA. Each of these methodologies has advantages and disadvantages. *In situ* hybridisation visualises transcript abundance of target genes in the entire brain section and thus provides a clear and comprehensive overview of which neural subpopulations may be of interest. The disadvantage of using ISH to quantify expression of neurobiological markers is that the quantification process lacks power in comparison to qPCR and RNA-seq. That is, in our analysis, the quantification process is binary: cells are either classified as expressing or non-expressing, while the relative transcript abundance within the cells is not considered. Furthermore, manual and even semi-automated counting of labelled cells is a time-consuming process and not suitable for large numbers of samples.

Compared to ISH, qPCR is a more efficient tool for the quantification of the total gene expression within tissues. However, when the aim is to assess gene expression within specific neural subpopulations, it can be a challenge to accurately isolate the tissue of these neural subpopulations, which we achieved by performing mechanical microdissections. With ISH, thin (14 μm) sections were made and the entire tissue was stained, making the neural subpopulations clearly visible. However, with the needle microdissections that we employed in **Paper II**, the sections were thicker (100 μm), the tissue was frozen and no staining was applied to outline neural populations, and consequently the subregions were less clearly defined within the frozen tissue. This can impede accurate identification and collection of the subregion tissue, and despite careful standardisation and employing a conservative approach by avoiding inclusion of any dubious areas, it is possible that either the entire area of interest is not fully dissected out, or that additional tissue is accidentally collected from outside the regions of interest. A way to overcome this caveat is to use laser-capture microdissection, where specific cells can be isolated from microscopic slides with great precision (Espina et al., 2006). We recommend that future studies implement this technique to increase the accuracy of the tissue isolation.

Finally, RNA-seq is a powerful technique which provides a quantitative insight into the entire transcriptome of the studied tissue and as such, it has a very broad scope. Once an efficient pipeline has been established, processing the bioinformatics on large volumes of samples is generally not very time-consuming, particularly considering how much data can be generated per sample. One of the disadvantages of RNA-seq is that it is an expensive technique, often costing several hundred Euros per sample. As such, it was not feasible in **Paper III** to conduct RNA-seq for all the telencephalic subregions separately, and we were forced to perform the analysis on whole telencephalon tissue in a few selected individuals. A visual representation of some of the advantages and disadvantages of these three methodologies, as they were employed in this thesis, is provided in **Figure 14**.

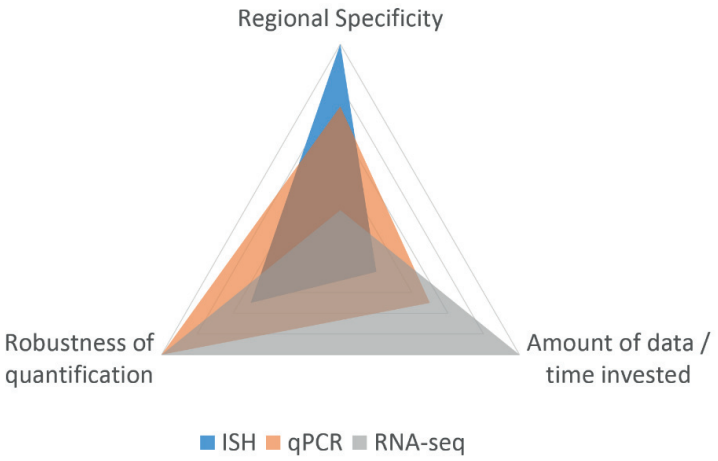

Figure 14. Schematic representation of the strengths and weaknesses of ISH, qPCR and RNA-seq, as used in this thesis, to assess expression of neural plasticity markers in the Atlantic salmon telencephalon. This representation only relates to the techniques as they were employed in this thesis and does not relate to the full potential of the techniques.

Finally, it is important to remark that all three methodologies used in this thesis visualise or quantify mRNA, while we did not have the resources to include corresponding protein analyses. Translation can be affected by many factors, such as secondary RNA structure, regulatory proteins and ribosome occupancy, and mRNA and protein levels are therefore not always correlated (Maier et al., 2009). Additionally, it is interesting to note that proteins can have both active and inactive isoforms, as is the case for the teleostean Bdnf protein, which occurs as an inactive precursor protein 'proBdnf', and as the functional mature protein 'Bdnf' (Tognoli et al., 2010). Therefore, it would be interesting to include protein-based analyses of neuroplasticity markers in future studies. To this end, immunohistochemistry can be used to identify neural subregions of interest and enzyme-linked immunosorbent assays (ELISA) and mass spectrometry technologies can be used to quantify (relative) protein abundance (e.g. Maier et al., 2009; Williams and Undieh, 2009).

4. Results: Summary of papers

Paper I – Neurobiology of wild and hatchery-reared Atlantic salmon: How nurture drives neuroplasticity

Life experiences in the rearing environment shape the neural and behavioural plasticity of animals. In fish stocking practices, the hatchery environment is relatively stimulus-deprived and does not optimally prepare fish for release into the wild. While the behavioural differences between wild and hatchery-reared fish have been examined to some extent, few studies have compared neurobiological characteristics between wild and hatchery-reared individuals in detail. Here, we compare the expression of immediate early gene *cfos* and neuroplasticity marker *brain derived neurotrophic factor* (*bdnf*) in telencephalic subregions associated with processing of stimuli in wild and hatchery-reared Atlantic salmon at basal and 30 min post (acute) stress conditions. Using *in situ* hybridization, we found that the expression level of these markers is highly specific per neuronal region and affected by both the origin of the fish, and exposure to acute stress. Expression of *cfos* was increased by stress in all brain regions and *cfos* was more highly expressed in the Dlv (functional equivalent to the mammalian hippocampus) of hatchery-reared compared to wild fish. Expression of *bdnf* was higher overall in hatchery fish, while acute stress upregulated *bdnf* in the Dm (functional equivalent to the mammalian amygdala) of wild, but not hatchery individuals. Our findings demonstrate that the hatchery environment affects neuroplasticity and neural activation in brain regions that are important for learning processes and stress reactivity, providing a neuronal foundation for the behavioural differences observed between wild and hatchery-reared fish.

Paper II – Effects of environmental enrichment on forebrain neural plasticity and survival success of stocked Atlantic salmon

In stocking programs, hatchery-reared fish are severely stimulus-deprived compared to their conspecifics in natural conditions, which leads to reduced behavioural plasticity and, consequently, low post-release survival rates of stocked fish. Structural environmental enrichment has been shown to have positive effects on life-skills which are critical to the survival of hatchery-reared fish, such as predator avoidance and foraging behaviour, but the neural mechanisms underpinning these behavioural changes are still largely unknown. In this study, juvenile Atlantic salmon (*Salmo salar*) were reared in an enriched hatchery environment for seven weeks, after which neurobiological characteristics and post-release survival were compared to fish reared under normal hatchery conditions. Using *in situ* hybridisation and qPCR, we quantified the expression of four neuroplasticity and neurogenesis markers in telencephalic subregions associated with relational memory, emotional learning, stress reactivity and goal-oriented behaviour. Aside from lower expression of *brain-derived neurotrophic factor* (*bdnf*) in the Dlv (a region associated with relational memory and spatial orientation) of enriched salmon, enrichment had no significant effects on neural plasticity. In December, following the 7-week treatment, a total of 627 fish, reared either in enriched or control tanks, were released into the wild, where fish remained for five months until downstream migration during the following spring. Exposure to an enriched environment increased post-release survival and subsequent successful downstream migration by 51%. Further, smolts in the mid-size range (110-170 mm) showed higher survival rates than both smaller (<110 mm) and larger (>170 mm) smolts. Thus, we demonstrate that environmental enrichment can improve stocking success of Atlantic salmon parr, but this does not appear to be associated with increased neural plasticity in the studied telencephalic subregions.

Paper III – Brain training: Can swimming exercise promote brain plasticity and cognition in Atlantic salmon?

It is well established that running exercise can enhance brain plasticity and boost cognitive performance in mammals, but this relationship has been little studied in fish. The aim of this study was to determine whether an 8-week sustained swimming exercise regime can enhance brain plasticity, cognition and foraging behaviour in juvenile Atlantic salmon. Fish were subjected to either a fixed flow rate of 3.5 BL s^{-1} ("forced exercise") or to a variable flow rate between 0.4 – 2.9 BL s^{-1}, where fish could choose their swimming speed ("volitional exercise"). Compared to non-exercised controls, exercised groups showed 27% (volitional) and 31% (forced) higher growth. To test cognition, volitional exercised and non-exercised controls were subjected to a repeated maze test. While both groups solved the maze more quickly over time, indicating a learning process, no significant difference was observed between exercised and non-exercised fish in the time taken to solve the maze. To study foraging behaviour, twenty exercised and twenty non-exercised fish were isolated in individual aquaria and presented with a novel prey (a live cricket) twice per day for five consecutive days. No differences in time to consumption and number of consumed prey were found between treatments. Brain plasticity was assessed through mapping the transcriptome of the telencephalon: the brain area specifically involved with learning, memory and decision making in teleosts. In the volitional exercise group, 1772 transcripts were differently expressed compared to unexercised control individuals. Several of these transcripts were related to molecular pathways and processes which are known to be involved in exercise-induced neuroplasticity in the mammalian hippocampus, such as synaptic trafficking, signal transduction and the glutamatergic and GABAergic systems. Gene ontology (GO) analysis identified 195 and 272 GO categories with a significant overrepresentation of up- or downregulated transcripts, respectively. A multitude of these GO categories related to neuronal excitability, neuronal signalling, cell proliferation and neurite outgrowth. In conclusion, sustained swimming exercise promoted the expression of neuroplasticity- and neurogenesis-related genes in the telencephalon, but did not have an apparent effect on the cognitive capacity of the fish in a spatial orientation test. Notably, this is the first time that swimming exercise has been directly linked to increased telencephalic neural plasticity in a teleost, and our results pave the way for future studies on exercise-induced neuroplasticity in fish.

5. Discussion

The results presented in this thesis describe the effects of the rearing environment on telencephalic neural plasticity and behavioural responses in juvenile Atlantic salmon, and discuss potential implications in relation to stocking programs. First, we characterised the expression of neuroplasticity markers in the telencephalon of wild and hatchery-reared Atlantic salmon within five neural populations important for cognitive processes – the most detailed characterisation to date. Second, we assessed how structural environmental enrichment affects the region-specific expression of these neural plasticity markers, and whether this may be linked to improved post-release survival. Third, the potential of swimming exercise as alternative rearing method to promote telencephalic neural plasticity and cognition in Atlantic salmon was evaluated.

5.1 Neurobiology of wild and hatchery-reared salmon

In order to rear more 'wild-like' fish under hatchery conditions, it is important that we first have a thorough understanding of how the neurobiological and behavioural characteristics of hatchery-reared fish differ from those of their wild conspecifics. However, these differences have not been thoroughly mapped, particularly regarding neurobiological characteristics. Specifically, very few studies have directly compared neurological traits between wild and hatchery-reared salmonids of the same genetic origin, and the few studies that have done so, have only quantified neuroplasticity markers either at the level of the whole brain (e.g. Aubin-Horth et al., 2005) or large brain structures such as the hind- and mid-brain (e.g. Dunlap et al., 2011). However, when studying such macro-brain areas, one studies a conglomerate of many different neural subregions, and regional nuances cannot be detected. Therefore, in **Paper I**, we set out to characterise the expression of *cfos* and *bdnf* in telencephalic subregions at the most detailed scale to date.

The first main finding of this study is that the quantification of neuroplasticity markers, using a region-specific approach, reveals expression patterns that may escape detection when studying macro-brain areas such as the entire telencephalon. Indeed, we found that the expression levels of *cfos* and *bdnf* varied greatly between regions, corroborating previous findings by Vindas et al. (2017). The subregions also showed high variability in their responsiveness to an acute confinement stress. For example, in the Dld of hatchery-reared individuals, exposure to an acute stressor increased the number of *cfos*-labelled cells 4-fold, while in the Vv, a 113-fold increase was observed. Thus, our findings demonstrate that the

telencephalic subregions are highly heterogeneous regarding their neural activation in response to stress. Moreover, in some cases, divergent expression patterns were observed between the ventral and dorsal subregions of the Dl and the Dm. For example, *cfos* showed origin, treatment and interaction effects in the Dlv, while the Dld showed only treatment effects. Furthermore, in the Dmd, wild stressed fish showed a significantly higher number of *bdnf*-labelled cells compared to fish at basal conditions, while this trend was not observed in the Dmv. Thus, our findings support the hypothesis that the Dl and Dm are both comprised of a ventral and dorsal subregion, each with a unique responsiveness to stimuli (Broglio et al., 2015; Broglio, pers. comm.). Our observations also shed more light on some apparently contradictory findings reported by other authors. For example, three studies which have assessed the effects of acute stress on the teleostean neural *bdnf* response report either increased *bdnf* expression in whole-brain tissue of stressed zebrafish (Pavlidis et al., 2015) or no effects on *bdnf* expression in whole-telencephalon samples of rainbow trout (Johansen et al., 2012) or in the whole brain of European sea bass *Dicentrarchus labrax* (Tognoli et al., 2010). In our study, wild salmon parr showed a significantly increased post-stress *bdnf* transcript abundance in the Dm, but not in the Dl or Vv, thus demonstrating that the *bdnf* response to acute stress is highly region specific. With such a heterogeneous *bdnf* transcription stress-response between neural subregions, it is not surprising that generalised whole-brain expression patterns may yield contradictory results. Thus, recent advancements in teleostean functional neurobiology have identified several telencephalic subregions which are associated with behavioural processes which are important for salmon post-stocking performance, and our findings show that by specifically targeting these neural regions, we can uncover neural trends that remain undetected in whole-brain studies. Notably, this finding has implications for EE studies, which to date have only studied neurological effects at the macro-brain scale (e.g. von Krogh et al., 2010; Salvanes et al., 2013). Therefore, we employ a region-specific approach in **Paper II**, where we study the effects of EE-rearing on salmon telencephalic neural plasticity.

The second main finding of **Paper I** is that wild and hatchery-reared Atlantic salmon parr show clear region-specific differences in neural activation and neurotrophin expression. Post-stress abundance of *cfos*-labelled cells was increased in all regions, but in the Dlv (the proposed functional equivalent to the mammalian hippocampus) of hatchery-reared individuals, the number of *cfos*-labelled cells increased 21-fold in response to stress, while in wild fish, only a 7-fold increase was observed. In mammals, the hippocampal *CFOS* response is important, among other things, in fear memory storage and retrieval (Liu et al., 2012). In

this light, it would be interesting to assess whether wild and hatchery-reared fish would also display differences in neural activation in the Dlv in a fear-conditioning test, and how this may relate to important behavioural paradigms such as antipredator behaviour. Second, hatchery-reared fish showed higher *bdnf* expression compared to wild fish in the Dlv, Dmv, Dmd and Vv, with the highest number of *bdnf*-labelled cells in the Dm, which is important for learning under fear and stress, among other things (Portavella et al., 2004; Vargas et al., 2009; O'Connell and Hofmann, 2011). Under standard hatchery conditions, fish regularly experience disturbances (e.g. tank cleaning, grading, vaccination, transport etc.) at unpredictable intervals. It is conceivable that these stressors periodically trigger *bdnf* transcription in the Dm of hatchery fish, effectively elevating basal *bdnf* expression levels in this subregion. Interestingly, the Dm is reciprocally connected to the Vv, which in turn connects to the Dlv (Folgueira et al., 2004a; b; Rink and Wullimann, 2004; Northcutt, 2006), and it is thus possible that the frequent disturbances associated with life in an anthropogenic environment (i.e. hatchery rearing) increase *bdnf* expression at basal conditions in the Dm, which in turn promotes *bdnf* expression in the Dl and Vv through neural circuits involved in the stress axis. Together with the fact that hatchery-reared fish did not show an increase in *bdnf* to stress, and the fact that increased BDNF levels are linked to a higher learning performance in mammals (Vaynman et al., 2004), our results indicate a potentially reduced capacity for learning performance in hatchery-reared fish under acute stressful conditions, which may affect their post-release survival, particularly in risky environments (e.g. under predator pressure).

In summary, with this initial characterization of telencephalic region-specific neural plasticity we have demonstrated that a region-specific approach reveals neural trends which remain undetected in whole-telencephalon studies, and we have characterised several differences in the expression of neuroplasticity markers between wild and hatchery-reared fish in neural regions which are important for stress reactivity, associative learning and fear memory and retrieval. With this study, we thus uncover, for the first time, several neural differences between wild and hatchery-reared fish, which may contribute to the reported behavioural differences between these two groups. Importantly, this study establishes a baseline from which we can investigate further how innovative rearing strategies such as EE (**Paper II**) may enhance the neurobiology of hatchery-reared fish, and produce a more "wild-like" reared salmon.

5.2 Environmental enrichment

Environmental enrichment is among the most studied forms of hatchery optimization for stocking programs (e.g. Johnsson et al., 2014; Näslund and Johnsson, 2014). Most studies have focused on the effects of EE on fish behaviour and stocking success, while effects on fish neurobiology have been investigated to a lesser extent. The studies which have assessed the effects of EE on fish neurobiology have focused on expression of markers for neurogenesis or neural plasticity within the entire telencephalon (e.g. von Krogh et al., 2010; Salvanes et al., 2013; Manuel et al., 2015), while no studies have ever investigated the effects of EE on the scale of neural subregions. After we had established in **Paper I** that a detailed telencephalic characterisation can uncover trends that may remain undetected in whole-telencephalon samples, we set out to determine the effects of a 7-week period of EE on telencephalic region-specific expression of the neural markers *cfos*, *bdnf*, *neurod* and *pcna*, as well as the freshwater post-release survival of Atlantic salmon parr in **Paper II**.

A seven-week exposure to an EE prior to release significantly improved post-release freshwater survival of stocked Atlantic salmon by 51%. It is a common stocking practice to release salmon smolts of large size, which have repeatedly been shown to have a higher survival probability than smaller-sized conspecifics (Kallio-Nyberg et al., 2004; Kallio-Nyberg et al., 2011; Rosengren et al., 2016). Large salmonids are often (Abbott et al., 1985; Johansen et al., 2012), though not always (Huntingford et al., 1990) reported to be dominant over smaller individuals, and dominant individuals may have competitive advantages over subordinate conspecifics (Metcalfe, 1986; Metcalfe et al., 1989). Furthermore, large salmon may be less prone to predation pressures (Skilbrei et al., 1994), thereby improving their survival probability. However, there is a trade-off between these advantages and increased metabolic demands which come with increased body size (Brett and Glass, 1973). In contrast to previous studies, we observed reduced freshwater survival rates for the largest size classes of stocked parr. This reduced freshwater survival of large stocked salmon is possibly linked to the relatively low food availability in the freshwater environment during the winter period, which is supported by the observation that only fish of intermediate size increased in body mass during their river residency. Studies which report increased survival with increasing body size have mostly monitored salmonid survival in seawater, where food is more abundant (e.g. Kallio-Nyberg et al., 2004; Kallio-Nyberg et al., 2011) or during a brief freshwater residency (Rosengren et al., 2016), when limited food availability does not have a large impact on fish survival, as they can live off their fat deposits. Our findings suggests that during a

prolonged freshwater residency, increased metabolic demands may hinder large-sized salmon, and intermediate-sized individuals may consequently have a competitive advantage over large individuals.

Despite a significant improvement of stocking success, we found few significant effects of EE on the studied neural markers following seven weeks of treatment. In fact, the only significant effect was found in the Dlv, where EE-exposed fish showed fewer *bdnf*-labelled cells compared to control fish. Interestingly, in **Paper I** we found that wild-caught salmon parr show a significantly lower number of *bdnf*-expressing cells in the Dlv, Dmd, Dmv and Vv, compared to hatchery-reared parr of the same genetic background. In this respect, the lower *bdnf* expression seen in the Dlv of enriched fish in the current study resembles the wild phenotype more closely than control fish. In **Paper I**, we hypothesised that higher *bdnf* expression in the Dlv of hatchery-reared salmon, compared to wild fish, may be linked to stressors in the hatchery environment. If this is extrapolated to the results obtained in **Paper II**, this suggests that a 7-week exposure to EE may have reduced stress in EE-reared fish, as has been shown in other studies (reviewed by Näslund and Johnsson, 2014). Aside from lower *bdnf* abundance in the Dlv of EE-reared fish, we found no differences in expression of any of the other markers in the Dld, Dlv, Dmd, Dmv or Vv. Additionally, we quantified the number of *cfos*- and *bdnf*-labelled cells (by ISH) in the dorsal nucleus of the ventral telencephalon (Vd) and the preoptic area of the hypothalamus (POA; **Fig. 15**). The Vd has been reported to share anatomical similarities with the mammalian striatum, and has also been proposed as a partial homolog to the nucleus accumbens (NAcc; O'Connell and Hofmann, 2011). While the functionality of the Vd has not yet been fully scrutinised, one of the proposed functions is that it may be involved in avoidance behaviour (Lau et al., 2011). The POA is located on the border of the telencephalon and hypothalamus, and in teleosts it has been reported to be involved in the regulation of different forms of stress, predominantly social stress (Doyon et al., 2003; Bernier and Craig, 2005; Doyon et al., 2005; O'Connell and Hofmann, 2011). In both the Vd and POA, we also found no differences in *cfos* and *bdnf* expression between EE and control groups (**Fig. 16**; unpublished data). In fact, we found no *bdnf*-labelled cells in the Vd of both groups, highlighting once again the regional specificity of *bdnf* expression. Our observation of the minimal effects of EE on neural plasticity markers in the targeted regions contrasts with other studies, which describe altered *pcna* and *neurod* expression in whole-telencephalon samples of EE-reared fish (von Krogh et al., 2010; Salvanes et al., 2013; Manuel et al., 2015). However, as these studies targeted the entire telencephalon, it is possible that the neural effects of EE might be manifested in subregions which were not included in

Paper II. Alternatively, the apparent lack of neurobiological effects in this study may be due to the duration of exposure or the time point of sampling. That is, a recent study has suggested that neural plasticity markers are upregulated in the telencephalon in the immediate weeks following transfer to an enriched holding tank, but that this expression decreases back to baseline levels within several weeks (L.O.E. Ebbesson, unpublished data).

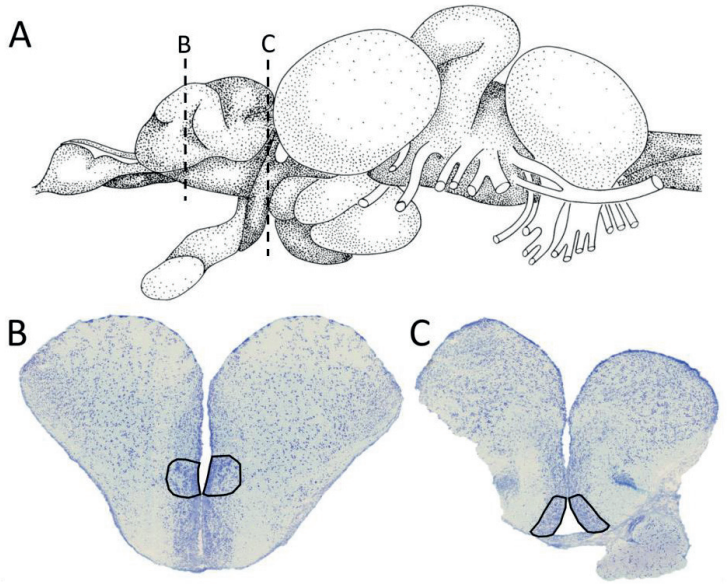

Figure 15. Location of the dorsal nucleus of the ventral telencephalon (Vd; **B**) and the preoptic area (POA; **C**).

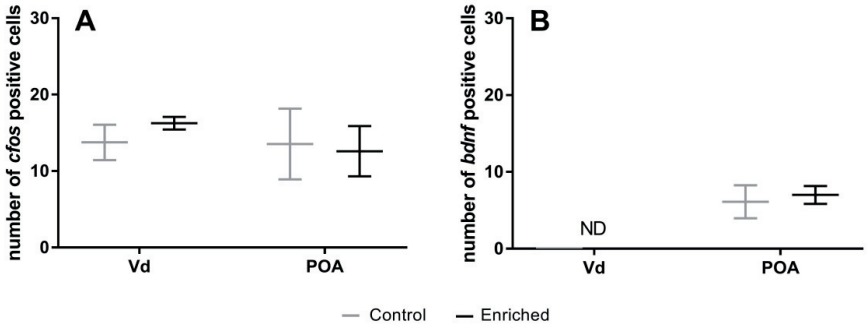

Figure 16. *In situ* hybridisation labelled *cfos* and *bdnf* cells in telencephalon subregions (unpublished data from **Paper II**). Mean ± SEM number of *cfos* (**A**) and *bdnf* (**B**) positive cells in the dorsal nucleus of the ventral telencephalon (Vd) and the preoptic area (POA) in Atlantic salmon parr after seven weeks in a control (grey bars) or enriched environment (black bars). For control animals, $n = 8$ per brain area, while for enriched animals, $n = 7$ per area. ND = not detected.

In both **Paper I** and **II** we quantified ISH-labelled *cfos* and *bdnf*-expressing cells in telencephalic subregions of hatchery-reared individuals at basal conditions. The fish used in these two papers were siblings and reared in the same hatchery, and with the absence of genetic and environmental differences, we can compare the ISH data between these groups, even though the fish in **Paper I** were sampled in September and the individuals in **Paper II** were sampled in November and there is thus a slight age difference. The ISH quantification was performed by two different experimenters between the two studies, but inter-specific observer bias was reduced as much as possible by adopting a semi-automated quantification procedure and by corroborating that the two experimenters showed a consistent methodology regarding area selection and threshold application. Between the two studies, we generally observed similar *cfos* and *bdnf* expression profiles among the telencephalic regions (**Fig. 17**), suggesting that the quantification methodology and results are quite robust. Interestingly, both *cfos* and *bdnf* appear to be lower expressed in the Dld of fish in **Paper II**. As outlined above, the age difference of the fish seems to be the most noticeable difference between fish in the two studies, which could explain this observation, but as no characterisation has been made of the telencephalic expression of these markers with age, we cannot conclude on this with certainty.

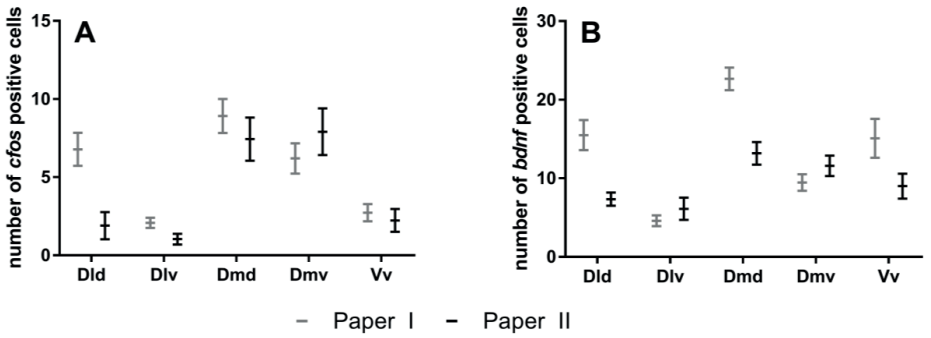

Figure 17. The number (mean ± SEM) of *cfos* (**A**) and *bdnf* (**B**) transcript-labelled cells in telenceaphalic subregions of hatchery-reared Atlantic salmon parr at basal conditions quantified in **Paper I** (grey bars) and **Paper II** (black bars).

As mentioned in **Section 1.3**, there are conflicting reports on the efficacy of EE in altering fish behaviour, neurobiology and post-release survival. These differences may be largely attributed to a lack of standardization, as the type of enrichment, age of the fish and exposure duration may all affect the outcome of EE (e.g. Lambert et al., 2005; Manuel et al., 2015; Ahlbeck Bergendahl et al., 2016). Further empirical testing of optimal EE conditions can shed light on inconsistencies between published enrichment studies, and contribute to optimisation of hatchery protocols.

Based on the results presented in **Paper II**, it appears that a 7-week exposure to EE, and selection for intermediate-sized individuals, may improve freshwater post-release survival of hatchery-reared Atlantic salmon, particularly when they are released several months prior to seawater migration. However, the effectiveness of EE in improving salmon stocking practices still remains controversial, as while some studies have shown that EE can lead to improved post-release survival (Maynard et al., 1995; Hyvärinen et al., 2013; Roberts et al., 2014), others have found no effects (Brockmark et al., 2007; Fast et al., 2008; Tatara et al., 2009; Brockmark and Johnsson, 2010) or even negative effects (Berejikian et al., 1999; Rosengren et al., 2016). Importantly, the majority of hatchery managers are reluctant to implement EE in their hatcheries, because the addition of structures to the tanks leads to waste accumulation and hinders fish monitoring. Thus, the efficacy of EE in improving stocking success remains debated, and there are practical concerns regarding the implementation of this procedure in hatcheries. Therefore, it is important to explore the potential of other innovative hatchery

protocols which may improve the stocking success of salmon. In **Paper III**, we assess the potential of one of these strategies, which is the application of sustained swimming exercise.

5.3 Swimming exercise

Captive animals are often restricted in their movement and consequently, they exercise less compared to their conspecifics in the wild. In captive mammals, running exercise has been shown to improve hippocampal neurogenesis, neural plasticity and cognition. Although preliminary studies suggest that exercise-enhanced neuroplasticity and cognition may also occur in teleosts (Luchiari and Chacon, 2013; Fiaz et al., 2014), this link has never been thoroughly studied in fish. Therefore, the aim of **Paper III** was to assess whether sustained swimming exercise can promote forebrain neuroplasticity and cognition in Atlantic salmon, which may open up the possibility of using swimming exercise to improve the fitness of hatchery-reared fish.

After eight weeks of sustained exercise at a volitional swimming speed (i.e. where individuals chose their swimming speed), juvenile salmon showed increased telencephalic neural plasticity compared to unexercised controls. Between the two groups, GO analysis revealed 195 and 272 GO categories with a significant overrepresentation of up- or downregulated transcripts, respectively. Several of these GO categories with an overrepresentation of upregulated genes were related to neural excitability, neuronal signalling, cell proliferation and neurite outgrowth, while categories with an overrepresentation of downregulated genes included several apoptosis-related GO categories. Thus, exercise at a volitional swimming speed affects the transcriptional pathways which regulate neural plasticity, cell proliferation, and cell survival in fish.

The molecular mechanisms which underlie exercise-enhanced neural plasticity are still disputed, but mammalian studies are starting to unravel the signalling pathways involved in exercise-enhanced neurogenesis and synaptogenesis (Molteni et al., 2002; Lista and Sorrentino, 2010). A schematic representation of this pathway is depicted in **Fig. 18**. In summary, physical activity leads to increased abundance of neurotrophins, such as BDNF and insulin-like growth factor (IGF; Vivar et al., 2013). Subsequently, BDNF can directly promote neurogenesis, or it may activate signal transduction pathways through signalling molecules such as calcium/calmodulin-dependent protein kinase II (CAMK-II), mitogen-activated protein kinase (MAPK), protein kinase C (PKC) and cAMP response element binding (CREB) protein (Molteni et al., 2002; Farmer et al., 2004), which in turn stimulate

synaptogenesis, long-term memory (LTM) and LTP (reviewed by Lista and Sorrentino, 2010). Furthermore, synaptogenesis is stimulated by synaptic trafficking molecules such as synaptotagmin and syntaxin, which are promoted through CAMK-II after activation by BDNF or IGF (Molteni et al., 2002). Interestingly, in our study, the telencephalic transcriptome of exercised Atlantic salmon revealed an upregulation of several transcripts involved in this pathway, such as the growth factor IGF, signal transduction molecules such as CAMK-II, MAPK, PKC and CREB, as well as the synaptic trafficking molecules synaptotagmin and syntaxin. These results suggest that the molecular pathways underlying exercise-enhanced neuroplasticity may be quite conserved between mammals and fish. Surprisingly, while we observed increased expression of many of these neuroplasticity-related signalling molecules in exercised fish, we did not observe an increased abundance of *bdnf* itself. Importantly, we only assessed the telencephalon transcriptome after eight weeks of swimming exercise, thus mapping the chronic effects of swimming. It is possible that telencephalic *bdnf* abundance increases immediately following the onset of swimming and subsequently decreases to basal levels again, as has been observed in salmon following transfer to a holding tank enriched with physical structures (L.O.E. Ebbesson, pers. comm.). To verify this possibility, future studies should perform a time series and quantify *bdnf* transcript levels at several hours, days and weeks after the onset of exercise.

Although different methodologies were used, it appears that eight weeks of sustained swimming exercise has a more pronounced effect on neural plasticity than seven weeks of exposure to EE, as reported in **Paper II**. However, in **Paper II** we used a region-specific approach while whole-telencephalon samples were studied in **Paper III**, and therefore comparisons between the two studies are hard to make. Using RNA-seq, **Paper III** has revealed that swimming exercise leads to an upregulation of several genes involved in (the molecular mechanisms underlying) neural plasticity, and it would be interesting to explore how exposure to EE would affect these markers (e.g. synaptic trafficking molecules and signalling molecules), and whether EE may activate the same neuroplasticity-related molecular pathways that we observe to be affected by swimming exercise.

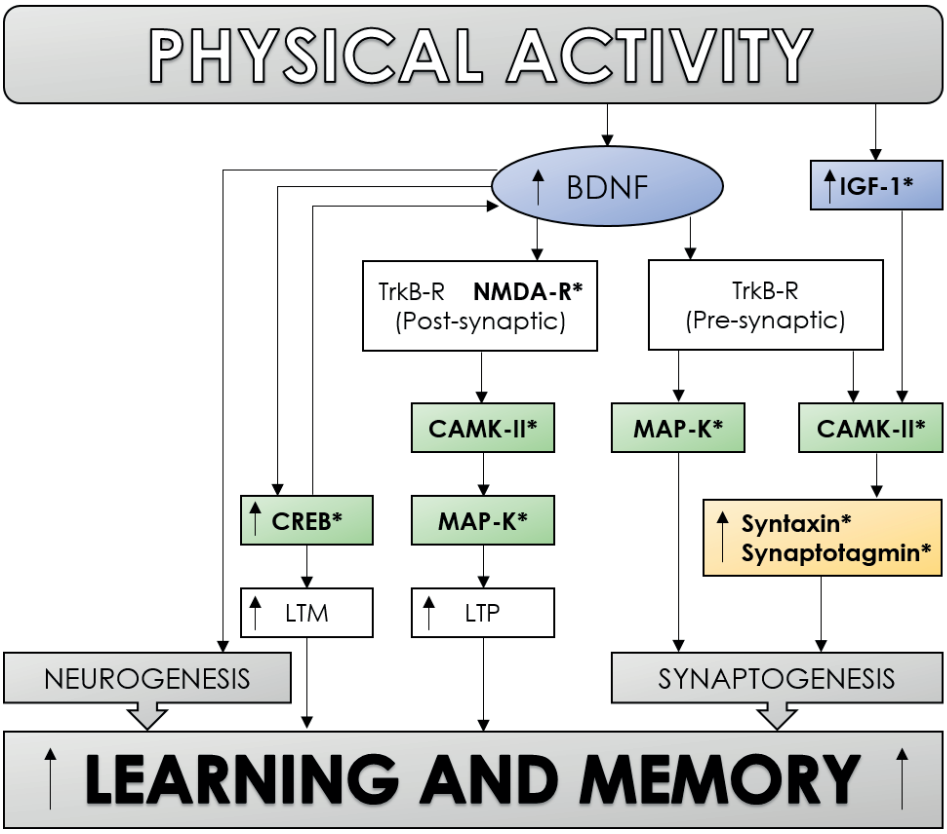

Figure 18. Proposed energy independent mechanisms through which physical activity may improve learning and memory in mammals. BDNF is a critical modulator of the energy independent effects of physical activity on neurogenesis and synaptogenesis. BDNF increases neurogenesis and activates complex presynaptic and postsynaptic molecular cascades that induce synaptogenesis. In this process, CAMK-II and MAP-K pathways seem to be fairly important acting on both LTP and LTM, the latter through the regulation of CREB levels. Physical activity also activates IGF-1 production that may lead to synaptogenesis through a downstream signaling cascade that at the presynaptic level includes CAMK-II and MAP-K. In exercised Atlantic salmon in **Paper III**, we found upregulated transcripts of several molecules involved in this pathway (bold molecules, marked with asterisk), compared to unexercised controls. Abbreviations: brain-derived neurotrophic factor (BDNF), Calcium/calmodulin protein kinase II (CAMK-II), cyclic adenosine monophosphate response element-binding (CREB), insulin-like growth factor (IGF-1), long-term memory (LTM), long-term potentiation (LTP), mitogen-activated protein kinase (MAPK), tyrosine kinase receptor B (TrKB-R). Modified from Lista and Sorrentino (2010).

In the volitional exercise group, we found 1772 transcripts which were significantly differently expressed (DE) compared to unexercised controls. Surprisingly, we found no significantly DE transcripts between the forced exercised group and their respective controls. It is plausible that this lack of a significant difference in gene expression between the forced exercise and control groups is a consequence of a smaller amplitude of the effects. In addition, we had to remove two outlier samples from the statistical analysis, resulting in a transcriptome comparison between 3 forced exercised and 5 respective control animals. This asymmetrical removal of outliers may have affected the statistical power in this comparison. Interestingly, despite the lack of significantly DE genes in the forced exercise and control groups, we observed a strong correlation trend in gene expression between volitional swimmers and exercised swimmers. That is, for the vast majority of genes significantly overexpressed in volitional swimmers, there was a non-significant overexpression in forced swimmers compared to forced controls. This suggests that both volitional and forced exercise exert a similar effect on the telencephalic transcriptome, even though the effects seem more pronounced and consistent in the volitional treatment. It would be interesting to determine why volitional exercise seems to have stronger beneficial effects compared to forced exercise, and we hypothesise that two factors may play an important role. First, the swimming speed in the forced treatment was close to U_{opt} for the average-sized fish, but fish inevitably varied in size. Consequently, the set swimming speed was relatively higher for the smallest size classes of fish, which may lead to differences in treatment effectiveness between fish size classes. The preferred swimming speed of salmonids tends to be close to their U_{opt} (Tudorache et al., 2011), and in the volitional treatment, fish of all sizes were allowed to position themselves in a flow rate that would not exceed their metabolic capacity or lead to stress. Second, fish in the volitional treatment were able to cease swimming occasionally, drifting down with the current, and this freedom to display an individually preferred behaviour may have potential positive welfare effects.

As the expression of neural markers can vary greatly between telencephalic subregions (**Papers I-II**), we would have ideally liked to perform RNA-seq analysis on the Dl, Dm and Vv separately. However, because RNA-seq is a costly methodology, we were forced to analyse whole-telencephalon samples in the current study. A disadvantage of this approach is that we do not have any insight into how exercise affects the individual telencephalic subregions, and in particular the Dl, which is associated with spatial memory and is thus important for the maze test performance of the fish (discussed below). However, we have collected additional whole-brain samples at three and eight weeks after the onset of

swimming, which may be used for future analyses. For example, as was conducted in **Paper II**, a targeted approach using microdissections and qPCR can be performed to corroborate the increased expression of genes which are involved in neuroplasticity pathways in exercised fish, as well as give more insight into the region-specific responses of the telencephalon to swimming exercise.

While we observed increased telencephalic expression of neuroplasticity markers in volitional exercised fish, we did not find any evidence of increased cognition of exercised individuals, as assessed in the maze test. This particular maze test was modelled after methodology described by Salvanes et al. (2013), and was selected because Salvanes and colleagues reported robust differences in maze performance between pre-smolt Atlantic salmon reared in either an enriched or barren environment, using F1 offspring from wild parents from a Norwegian river. However, we remark that this specific protocol involves considerable handling and air exposure of the fish prior to testing, which inadvertently causes stress and may negatively affect the cognitive performance of the animals. Furthermore, adverse effects of handling may be particularly pronounced in F1 offspring from wild parents, compared to highly domesticated fish species such as zebrafish, or even rainbow trout. We thus suggest that future studies employ cognitive tests which require less handling, or use a fish model which is more robust to handling stress in order to corroborate the possible implications of swimming exercise on cognitive capacity.

A final remark regarding the maze test is that we observed substantial personality differences between individual fish. Personality traits (e.g. coping styles) refer to consistent individual differences in behavioural responses, such as the shyness-boldness continuum (Coleman and Wilson, 1998). During the maze test, we scored the time to emergence from the start box in all individuals, and this measure effectively resembles the emergence test which is used to quantify boldness in fish (Brown and Braithwaite, 2004; Lee and Berejikian, 2008). We observed that some individuals were consistently quick to emerge (bold individuals), while others were consistently slow (shy individuals; **Fig. 19**). As variation in personality traits may affect behavioural responses, it is important to keep personality in mind as confounding factor in future cognitive studies.

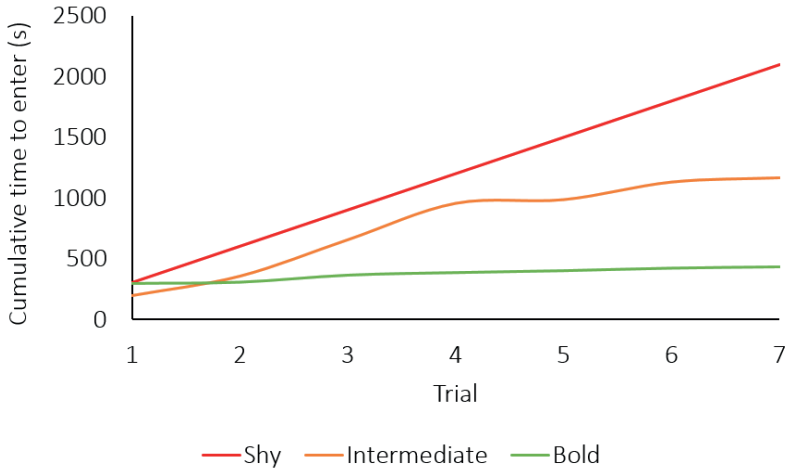

Figure 19. Examples of personality differences in the maze test. Depicted is the cumulative time to enter the maze from the start box after opening the hatch over seven trials, for three selected volitional exercised salmon. A shy fish (red) used the maximum time allowance of 300 s every trial, while a bold fish (green) entered the maze almost instantly every day, with the exception of day 1. Some fish showed an intermediate personality (orange), displaying shy behaviour during the first trials, but behaving more boldly as the trials progressed.

Aside from the maze test, we wanted to perform a cognitive test with a more applied purpose for stocking practices, i.e. a behavioural test which might be directly relevant for post-release survival. Therefore, we also subjected fish to a novel prey test, based on methodology described by Sundström and Johnsson (2001). In short, a group of volitional exercised fish and their respective controls (n = 20 per group) were placed in individual aquaria, and after a ten-day acclimatization period, fish were fed one live cricket (*Acheta domesticus*; body size 5.8 ± 7 mm; mean ± SD) during ten trials over five days, with two trials per day. For this experiment, a floating cricket was chosen because Atlantic salmon parr are known to readily consume surface-drifting prey (e.g. Johnson et al., 1996; Johansen et al., 2011) and hatchery-reared salmonids have an increased preference for drifting prey compared to wild conspecifics (e.g. Johnson et al., 1996; Teixeira and Cortes, 2006). For each trial, one live cricket was placed on the water surface for a maximum duration of 3 min, and if the cricket was not consumed within this time, the insect was removed from the aquarium. Time to first bite and the number of consumed prey were recorded. No pronounced differences in number of crickets consumed or time to first bite were found between exercised and control

animals (**Fig. 20**; unpublished data). Surprisingly, despite the fact that fish were not fed any additional feed during the trials, the highest observed percentage of fish that consumed their prey during a single trial was only 18% (7 out of 40 fish). These consumption rates are substantially lower than the consumption rates reported for hatchery-reared fish in the study by Sundström and Johnsson (2001), which ranged between 40-60% after six feeding trials. Notably, Sundström and Johnsson (2001) used brown trout in their study, which are known to be more resilient to stress compared to Atlantic salmon (Ø. Øverli, pers. comm.). Transfer to a new environment and social isolation can induce stress in salmonids and may lead to stress-induced anorexia in fish, the effects of which seem to be modulated by personality (Pickering et al., 1982; Øverli et al., 1998; Höglund et al., 2007; Basic et al., 2012). In this context, we believe that the salmon in the novel prey assay in **Paper III** were stressed from the housing conditions and that this affected their performance in the novel prey test. In other words, the low success rates of food consumption in the novel prey test were due to stress-induced anorexia and this was not affected by treatment (i.e. swimming exercise). Interestingly, stress-induced anorexia, related to handling and release procedures, may also contribute to observed low post-release feed intake in stocked fish, immediately after release (Munakata et al., 2000). Thus, hatchery techniques which can increase the resilience of hatchery-reared fish to stress-induced anorexia could have large implications for stocking programs, but we did not observe any evidence for increased resilience to stress-induced anorexia as a result of exercise training in this study.

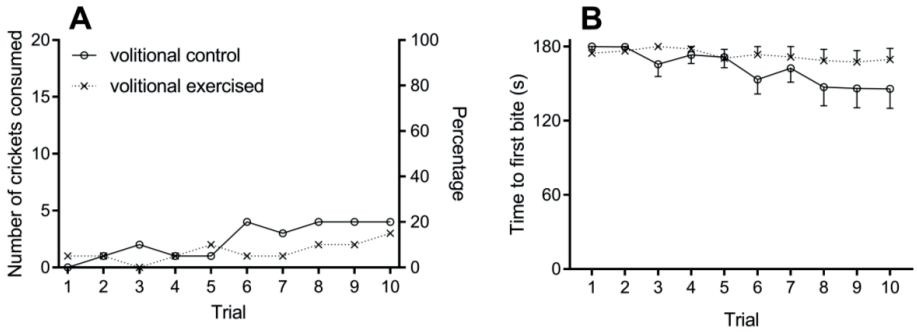

Figure 20. Volitional exercised fish and their respective controls (n = 20 per group) showed no clear differences in number of consumed crickets per trial (**A**) or time to first bite (**B**). Error bars depict SEM. Unpublished data from **Paper III**.

Aside from an apparent stimulatory effect of exercise on neural plasticity, exercise is also known to improve several production and welfare aspects in captive fish. For example, it has been demonstrated that swimming exercise can increase growth rates (reviewed by Davison and Herbert, 2013), improve feed conversion ratios (Leon, 1986; Davison, 1997; Palstra et al., 2015), reduce stress and agonistic behaviour (reviewed by Huntingford and Kadri, 2013), delay (Palstra et al., 2010; Graziano et al., 2018) or promote (Mes et al., 2016) sexual maturation, increase disease resistance (Castro et al., 2011) and boost swimming performance (Farrell et al., 1990). Interestingly, several studies have previously investigated the effect of swimming exercise on post-release survival rates of stocked salmon (Burrows, 1969; Lagasse et al., 1980; Cresswell and Williams, 1983; Leon, 1986; Evenson and Ewing, 1993; Hoffnagle et al., 2006) and most studies found a positive effect, provided that the fish were exercised at a moderate to high flow (reviewed by Maynard et al., 1995). Importantly, many salmon hatcheries rear fish in cylindrical tanks and thus, simply by manipulating the direction and volume of the water inflow, hatcheries can easily implement exercise regimes in their rearing practices. In summary, swimming exercise is relatively simple to implement in existing hatcheries, brings many physiological benefits and may also promote neuroplasticity in salmonids. Therefore, exercise appears to be a promising tool to improve stocking success in salmon.

6. Conclusions

This thesis contributes to an increased understanding of how the hatchery environment affects telencephalic neural plasticity of Atlantic salmon reared for stocking programs. It shows that, compared to wild conspecifics, salmon reared under traditional uniform rearing conditions display deviating neural activation and neurotrophin expression patterns in telencephalic subregions which are important for cognitive processes such as associative learning, stress reactivity and emotional learning. Our results provide the first detailed characterisation of neurological differences between wild and hatchery-reared Atlantic salmon, uncovering potential neural foundations to the behavioural differences which are observed between wild and hatchery-reared fish. Having established in more detail how hatchery-reared fish differ neurologically from wild conspecifics, our research sets a benchmark for future studies on hatchery optimisation and its implications on fish neurobiology and behaviour.

We found that exposure to EE can improve the post-release survival of reared salmon, although this did not seem to be associated with increased expression of telencephalic neural plasticity markers in the studied regions. Our findings suggest that when fish are stocked at the parr stage, freshwater survival and migration success can be improved by a short exposure to EE prior to release. While it has been reported that post-release survival increases with body size when salmon are stocked at the smolt stage, we found that when parr are stocked for a six-month freshwater period prior to spring smoltification, intermediate-sized fish show higher survival rates than the largest individuals, which has ramifications for parr stocking practices. We remark that the efficacy of EE in increasing salmonid stocking success is inconsistent among studies and that there are practical and hygienic concerns regarding implementation of EE in hatcheries. It is therefore imperative that we identify and evaluate alternative hatchery strategies which can improve salmon quality in stocking programs.

We propose that voluntary swimming exercise should be considered as alternative rearing strategy for hatcheries, as we find increased telencephalic expression of neural plasticity markers in exercised salmon parr. Implementation of swimming exercise does not interfere with hatchery operations and has been shown to have several additional production and welfare benefits such as improved growth efficiency and stress mitigation. Furthermore, exercise has already been shown in several cases to improve the post-release survival of fish, and we have now demonstrated that increased neural plasticity may be one of the underlying mechanisms behind this phenomenon. Thus, further validation of our findings can uncover

numerous potential applications for swimming exercise in neurological research, fish welfare and fish stocking practices.

7. Future perspectives

This thesis has identified and discussed several knowledge gaps which should be addressed in future studies. The main issues which require further investigation concern the resolution of teleostean neurobiological studies, optimisation and standardisation of experimental setups, and a more detailed characterisation of the neurological and cognitive effects of swimming exercise in fish.

First, we have corroborated that there is a large variability between telencephalic subregions regarding their neural activation and neurotrophic expression levels in response to environmental stimuli. While nearly all mammalian literature conducts neurological research on a highly detailed scale, it is still common in fish studies to quantify general measures such as brain size or whole-brain transcript abundance. To gain greater insight into the functional implications of teleostean neurobiological research, future studies should emphasise a more detailed approach focusing on distinct neural populations, which will provide a better understanding of how neurological characteristics are linked to fish behaviour.

Regarding EE, both the scientific and stocking community could benefit from studies which determine the optimal conditions of EE to promote fish welfare, development and fitness. This could firstly be done by reviewing the current literature in order to deduce which EE characteristics are associated with treatment effects. Second, empirical studies should aim to elucidate how characteristics such as age of the fish, treatment duration and type of enrichment affect the neurological and behavioural characteristics of the fish. Moreover, it should be investigated further whether critical 'opportunity windows' exist during early life, where exposure to EE can have lasting positive effects on fish development, preparing them for a future life in a natural environment. Such studies can provide researchers and hatchery managers with a scientific foundation to standardise and optimise their EE protocols, which will lead to improved stocking efficiency and facilitate the comparison of results between studies.

Finally, this thesis has provided the first evidence that swimming exercise can promote telencephalic neural plasticity in Atlantic salmon. Future studies should corroborate our findings and use a more specific approach, which targets the expression of neurotrophins and cell-proliferation markers in neural populations which are associated with cognition. As with EE, it is important to determine the optimal conditions for exercise-induced neural plasticity, for example by finding the optimal swimming speed and duration for cognitive stimulation. Additionally, possible critical time windows for exercise-enhanced neural plasticity may be

identified by establishing a temporal profile of neural plasticity marker expression following the onset of swimming. An important application of mammalian exercise-induced neuroplasticity research is its potential to prevent cognitive decline, particularly in the context of neurodegenerative diseases, such as Alzheimer's (reviewed by Ma et al., 2017). Compared to rodents, the use of teleost model species such as zebrafish has many advantages, such as low housing costs, short generation cycles and fewer ethical concerns (Lieschke and Currie, 2007; Kalueff et al., 2014). Therefore, zebrafish is becoming an increasingly popular model to study neurodegenerative diseases (Santana et al., 2012; Newman et al., 2014). Our finding that swim training can increase expression of cell proliferation and neurogenesis markers in the fish brain, and that the molecular pathways underlying exercised-enhanced neural plasticity appear to be quite conserved between fish and mammals, suggests that model fish species such as the zebrafish are a promising new animal model for exercise-induced neuroplasticity research.

8. References

Abbott, J.C., Dunbrack, R.L., and Orr, C.D. (1985). The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). *Behaviour* 92(3/4)**,** 241-253.

Ahlbeck Bergendahl, I., Salvanes, A.G.V., and Braithwaite, V.A. (2016). Determining the effects of duration and recency of exposure to environmental enrichment. *Appl. Anim. Behav. Sci.* 176**,** 163-169. doi: 10.1016/j.applanim.2015.11.002.

Amoroso, R.O., Tillotson, M.D., and Hilborn, R. (2017). Measuring the net biological impact of fisheries enhancement: Pink salmon hatcheries can increase yield, but with apparent costs to wild populations. *Can. J. Fish. Aquat. Sci.* 74(8)**,** 1233-1242. doi: 10.1139/cjfas-2016-0334.

Andersen, C.L., Jensen, J.L., and Ørntoft, T.F. (2004). Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res.* 64(15)**,** 5245. doi: 10.1158/0008-5472.CAN-04-0496.

Aprahamian, M.W., Martin Smith, K., McGinnity, P., McKelvey, S., and Taylor, J. (2003). Restocking of salmonids—opportunities and limitations. *Fish. Res.* 62(2)**,** 211-227. doi: 10.1016/s0165-7836(02)00163-7.

Araki, H., Berejikian, B.A., Ford, M.J., and Blouin, M.S. (2008). Fitness of hatchery-reared salmonids in the wild. *Evol. Appl.* 1(2)**,** 342-355. doi: 10.1111/j.1752-4571.2008.00026.x.

Araki, H., and Schmid, C. (2010). Is hatchery stocking a help or harm? *Aquaculture* 308**,** S2-S11. doi: 10.1016/j.aquaculture.2010.05.036.

Armstrong, J.D., Kemp, P.S., Kennedy, G.J.A., Ladle, M., and Milner, N.J. (2003). Habitat requirements of Atlantic salmon and brown trout in rivers and streams. *Fish. Res.* 62(2)**,** 143-170. doi: 10.1016/s0165-7836(02)00160-1.

Aubin-Horth, N., Letcher, B.H., and Hofmann, H.A. (2005). Interaction of rearing environment and reproductive tactic on gene expression profiles in Atlantic salmon. *J. Hered.* 96(3)**,** 261-278. doi: 10.1093/jhered/esi030.

Barker, J., M., Boonstra, R., and Wojtowicz, J.M. (2011). From pattern to purpose: How comparative studies contribute to understanding the function of adult neurogenesis. *Eur. J. Neurosci.* 34(6)**,** 963-977. doi: 10.1111/j.1460-9568.2011.07823.x.

Bartley, D.M., and Bell, J.D. (2008). Restocking, stock enhancement, and sea ranching: Arenas of progress. *Rev. Fish. Sci.* 16(1-3)**,** 357-365. doi: 10.1080/10641260701678058.

Basic, D., Schjolden, J., Krogdahl, A., von Krogh, K., Hillestad, M., Winberg, S., et al. (2013). Changes in regional brain monoaminergic activity and temporary down-regulation in stress response from dietary supplementation with l-tryptophan in Atlantic cod (*Gadus morhua*). *Br. J. Nutr.* 109(12)**,** 2166-2174. doi: 10.1017/S0007114512004345.

Basic, D., Winberg, S., Schjolden, J., Krogdahl, Å., and Höglund, E. (2012). Context-dependent responses to novelty in Rainbow trout (*Oncorhynchus mykiss*), selected for high and low post-stress cortisol responsiveness. *Physiol. Behav.* 105(5)**,** 1175-1181. doi: 10.1016/j.physbeh.2011.12.021.

Bell, J.D., Leber, K.M., Blankenship, H.L., Loneragan, N.R., and Masuda, R. (2008). A new era for restocking, stock enhancement and sea ranching of coastal fisheries resources. *Rev. Fish. Sci.* 16(1-3)**,** 1-9. doi: 10.1080/10641260701776951.

Berejikian, B.A. (2005). Rearing in enriched hatchery tanks improves dorsal fin quality of juvenile steelhead. *N. Am. J. Aquacult.* 67(4)**,** 289-293. doi: 10.1577/A05-002.1.

Berejikian, B.A., Smith, R.J.F., Tezak, E.P., Schroder, S.L., and Knudsen, C.M. (1999). Chemical alarm signals and complex hatchery rearing habitats affect antipredator

behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. *Can. J. Fish. Aquat. Sci.* 56(5)**,** 830-838. doi: 10.1139/f99-010.

Bergesen, K.A., Pettersen, K., Larsen, B.M., and Johnsen, S. (2017). "Nina Forskningsstation, Ims. Årsmelding 2016".

Bernier, N.J., and Craig, P.M. (2005). CRF-related peptides contribute to stress response and regulation of appetite in hypoxic rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289(4)**,** R982-R990. doi: 10.1152/ajpregu.00668.2004.

Bernstein, J.J. (1970). "Anatomy and physiology of the central nervous system" in *Fish Physiology,* eds. W.S. Hoar & D.J. Randall. Academic Press, 1-90.

Bohlin, T., Hamrin, S., Heggberget, T.G., Rasmussen, G., and Saltveit, S.J. (1989). Electrofishing - Theory and practice with special emphasis on salmonids. *Hydrobiologia* 173(1)**,** 9-43. doi: 10.1007/BF00008596.

Brett, J.R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can.* 21(5)**,** 1183-1226. doi: 10.1139/f64-103.

Brett, J.R., and Glass, N.R. (1973). Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. *J. Fish. Res. Board Can.* 30(3)**,** 379-387. doi: 10.1139/f73-068.

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. B* 277(1696)**,** 3035-3043. doi: 10.1098/rspb.2010.0561.

Brockmark, S., and Johnsson, J.I. (2010). Reduced hatchery rearing density increases social dominance, postrelease growth, and survival in brown trout (*Salmo trutta*). *Can. J. Fish. Aquat. Sci.* 67(2)**,** 288-295. doi: 10.1139/F09-185.

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.

Broglio, C., Gómez, A., Durán, E., Ocaña, F.M., Jiménez-Moya, F., Rodríguez, F., et al. (2005). Hallmarks of a common forebrain vertebrate plan: Specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. *Brain Res. Bull.* 66(4)**,** 277-281. doi: 10.1016/j.brainresbull.2005.03.021.

Broglio, C., Gómez, A., Durán, E., Salas, C., and Rodríguez, F. (2011). "Brain and cognition in teleost fish" in *Fish Cognition and Behavior,* eds. C. Brown, K. Laland & J. Krause. Wiley-Blackwell.

Broglio, C., Martín-Monzón, I., Ocaña, F., Gómez, A., Durán, E., Salas, C., et al. (2015). Hippocampal pallium and map-like memories through vertebrate evolution. *J. Behav. Brain. Sci* 5(3)**,** 109-120. doi: 10.4236/jbbs.2015.53011.

Brown, C. (2001). Social learning and life skills training for hatchery reared fish. *J. Fish Biol.* 59(3)**,** 471-493. doi: 10.1111/j.1095-8649.2001.tb02354.x.

Brown, C., and Braithwaite, V.A. (2004). Size matters: A test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Anim. Behav.* 68(6)**,** 1325-1329. doi: 10.1016/j.anbehav.2004.04.004.

Brown, C., Davidson, T., and Laland, K. (2003). Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. *J. Fish Biol.* 63**,** 187-196. doi: 10.1046/j.1095-8649.2003.00208.x.

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

Brown, C., Laland, K., and Krause, J. (2006). "Fish cognition and behavior" in *Fish Cognition and Behavior,* eds. C. Brown, K. Laland & J. Krause. Blackwell Publishing, 1-8.

Brown, G.E., Ferrari, M.C.O., and Chivers, D.P. (2011). "Learning about danger: Chemical alarm cues and threat-sensitive assessment of predation risk by fishes" in *Fish Cognition and Behavior,* eds. C. Brown, K. Laland & J. Krause. Wiley-Blackwell.

Brown, G.E., and Smith, R.J.F. (1998). Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery-reared fish to recognize chemical cues of a predator. *Can. J. Fish. Aquat. Sci.* 55(611-617). doi: 10.1139/f97-261.

Bshary, R., and Brown, C. (2014). Fish cognition. *Curr. Biol.* 24(19)**,** R947-R950. doi: 10.1016/j.cub.2014.08.043.

Burrows, R.E. (1969). The influence of fingerling quality on adult salmon survivals. *Trans. Am. Fish. Soc.* 98(4)**,** 777-784. doi: 10.1577/1548-8659(1969)98[777:TIOFQO]2.0.CO;2.

Bustin, S.A. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J. Mol. Endocrinol.* 25(2)**,** 169-193. doi: 10.1677/jme.0.0250169.

Bustin, S.A. (2002). Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *J. Mol. Endocrinol.* 29(1)**,** 23-39. doi: 10.1677/jme.0.0290023.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., et al. (2009). The MIQE guidelines: *M*inimum *I*nformation for publication of *Q*uantitative real-time PCR *E*xperiments. *Clin. Chem.* 55(4)**,** 611-622. doi: 10.1373/clinchem.2008.112797.

Castro, V., Grisdale-Helland, B., Helland, S.J., Kristensen, T., Jørgensen, S.M., Helgerud, J., et al. (2011). Aerobic training stimulates growth and promotes disease resistance in Atlantic salmon (*Salmo salar*). *Comp. Biochem. Physiol. A* 160(2)**,** 278-290. doi: 10.1016/j.cbpa.2011.06.013.

Chaput, G. (2012). Overview of the status of Atlantic salmon (*Salmo salar*) in the North Atlantic and trends in marine mortality. *ICES J. Mar. Sci.* 69(9)**,** 1538-1548. doi: 10.1093/icesjms/fss013.

Chung, A.-Y., Kim, S., Kim, H., Bae, Y.-K., and Park, H.-C. (2011). Microarray screening for genes involved in oligodendrocyte differentiation in the zebrafish CNS. *Exp. Neurobiol.* 20(2)**,** 85-91. doi: 10.5607/en.2011.20.2.85.

Clelland, C.D., Choi, M., Romberg, C., Clemenson, G.D., Fragniere, A., Tyers, P., et al. (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* 325(5937)**,** 210-213. doi: 10.1126/science.1173215.

Coleman, K., and Wilson, D.S. (1998). Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. *Anim. Behav.* 56(4)**,** 927-936. doi: 10.1006/anbe.1998.0852.

Cowx, I.G. (1994). Stocking strategies. *Fish. Manage. Ecol.* 1(1)**,** 15-30. doi: 10.1111/j.1365-2400.1970.tb00003.x.

Cresswell, R.C., and Williams, R. (1983). Post-stocking movements and recapture of hatchery-reared trout released into flowing waters - effect of prior acclimation to flow. *J. Fish Biol.* 23(3)**,** 265-276. doi: 10.1111/j.1095-8649.1983.tb02904.x.

Cross, T.F., McGinnity, P., Coughlan, J., Dillane, E., Ferguson, A., Koljonen, M.L., et al. (2007). "Stocking and ranching" in *The Atlantic Salmon: Genetics, Conservation and Management,* eds. E. Verspoor, L. Stradmeyer & J. Nielsen. Blackwell Publishing, 325-356.

Czerniawski, R., Pilecka-Rapacz, M., and Domagala, J. (2011). Stocking experiment with Atlantic salmon and sea trout parr reared on either live prey or a pellet diet. *J. Appl. Ichthyol.* 27(4)**,** 984-989. doi: 10.1111/j.1439-0426.2011.01761.x.

Davison, W. (1997). The effects of exercise training on teleost fish, a review of recent literature. *Comp. Biochem. Physiol. A* 117(1), 67-75. doi: 10.1016/S0300-9629(96)00284-8.

Davison, W., and Herbert, N.A. (2013). "Swimming-enhanced growth" in *Swimming physiology of fish: Towards using exercise to farm a fit fish in sustainable aquaculture,* eds. P.A. Palstra & V.J. Planas. (Berlin: Springer Berlin Heidelberg), 177-202.

Domenici, P., Steffensen, J.F., and Batty, R.S. (2000). The effect of progressive hypoxia on swimming activity and schooling in Atlantic herring. *J. Fish Biol.* 57(6), 1526-1538. doi: 10.1111/j.1095-8649.2000.tb02229.x.

Doyon, C., Gilmour, K.M., Trudeau, V.L., and Moon, T.W. (2003). Corticotropin-releasing factor and neuropeptide Y mRNA levels are elevated in the preoptic area of socially subordinate rainbow trout. *Gen. Comp. Endocrinol.* 133(2), 260-271. doi: 10.1016/S0016-6480(03)00195-3.

Doyon, C., Trudeau, V.L., and Moon, T.W. (2005). Stress elevates corticotropin-releasing factor (CRF) and CRF-binding protein mRNA levels in rainbow trout (*Oncorhynchus mykiss*). *J. Endocrinol.* 186(1), 123-130. doi: 10.1677/joe.1.06142.

Dunlap, K.D., Silva, A.C., and Chung, M. (2011). Environmental complexity, seasonality and brain cell proliferation in a weakly electric fish, *Brachyhypopomus gauderio*. *J. Exp. Biol.* 214(5), 794-805. doi: 10.1242/jeb.051037.

Ebbesson, L.O., and Braithwaite, V.A. (2012). Environmental effects on fish neural plasticity and cognition. *J. Fish Biol.* 81(7), 2151-2174. doi: 10.1111/j.1095-8649.2012.03486.x.

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.

Eisch, A.J., and Mandyam, C.D. (2007). Adult neurogenesis: Can analysis of cell cycle proteins move us beyond BrdU? *Curr. Pharm. Biotechnol.* 8(3), 147-165. doi: 10.2174/138920107780906540.

Espina, V., Wulfkuhle, J.D., Calvert, V.S., VanMeter, A., Zhou, W., Coukos, G., et al. (2006). Laser-capture microdissection. *Nat. Protoc.* 1, 586-603. doi: 10.1038/nprot.2006.85.

Evenson, M.D., and Ewing, R.D. (1993). Effect of exercise of juvenile winter steelhead on adult returns to Cole rivers hatchery, Oregon. *Prog. Fish Cult.* 55(3), 180-183. doi: 10.1577/1548-8640(1993)055<0180:EOEOJW>2.3.CO;2.

Farmer, J., Zhao, X., van Praag, H., Wodtke, K., Gage, F.H., and Christie, B.R. (2004). Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male sprague–dawley rats *in vivo*. *Neuroscience* 124(1), 71-79. doi: 10.1016/j.neuroscience.2003.09.029.

Farrell, A.P., Johansen, J.A., Steffensen, J.F., Moyes, C.D., West, T.G., and Suarez, R.K. (1990). Effects of exercise training and coronary ablation on swimming performance, heart size, and cardiac enzymes in rainbow trout, *Oncorhynchus mykiss*. *Can. J. Zool.* 68(6), 1174-1179. doi: 10.1139/z90-174.

Fast, D.E., Neeley, D., Lind, D.T., Johnston, M.V., Strom, C.R., Bosch, W.J., et al. (2008). Survival comparison of spring Chinook salmon reared in a production hatchery under optimum conventional and seminatural conditions. *Trans. Am. Fish. Soc.* 137(5), 1507-1518. doi: 10.1577/t07-143.1.

Fiaz, A.W., Léon-Kloosterziel, K.M., van Leeuwen, J.L., and Kranenbarg, S. (2014). Exploring the effect of exercise on the transcriptome of zebrafish larvae (*Danio rerio*). *J. Appl. Ichthyol.* 30(4), 728-739. doi: 10.1111/jai.12509.

Finstad, B., and Jonsson, N. (2001). Factors influencing the yield of smolt releases in Norway. *Nord. J. Freshwat. Res.* 75, 37-55.

Fischer, A. (2016). Environmental enrichment as a method to improve cognitive function. What can we learn from animal models? *NeuroImage* 131**,** 42-47. doi: 10.1016/j.neuroimage.2015.11.039.

Folgueira, M., Anadón, R., and Yáñez, J. (2004a). An experimental study of the connections of the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). I: Olfactory bulb and ventral area. *J. Comp. Neurol.* 480(2)**,** 180-203. doi: 10.1002/cne.20340.

Folgueira, M., Anadón, R., and Yáñez, J. (2004b). Experimental study of the connections of the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). II: Dorsal area and preoptic region. *J. Comp. Neurol.* 480(2)**,** 204-233. doi: 10.1002/cne.20341.

Fordyce, D.E., and Farrar, R.P. (1991). Enhancement of spatial learning in F344 rats by physical activity and related learning-associated alterations in hippocampal and cortical cholinergic functioning. *Behav. Brain Res.* 46(2)**,** 123-133. doi: 10.1016/S0166-4328(05)80105-6.

Fordyce, D.E., and Wehner, J.M. (1993). Physical activity enhances spatial learning performance with an associated alteration in hippocampal protein kinase C activity in C57BL/6 and DBA/2 mice. *Brain Res.* 619(1)**,** 111-119. doi: 10.1016/0006-8993(93)91602-O.

García de Leániz, C., Fleming, I.A., Einum, S., Verspoor, E., Consuegra, S., Jordan, W.C., et al. (2007). "Local adaptation" in *The Atlantic Salmon,* eds. Ø. Aas, S. Einum, A. Klemetsen & J. Skurdal. Wiley-Blackwell.

Gonda, A., Herczeg, G., and Merilä, J. (2009). Habitat-dependent and -independent plastic responses to social environment in the nine-spined stickleback (*Pungitius pungitius*) brain. *Proc. R. Soc. B*. doi: 10.1098/rspb.2009.0026.

Goodson, J.L., and Kingsbury, M.A. (2013). What's in a name? Considerations of homologies and nomenclature for vertebrate social behavior networks. *Horm. Behav.* 64(1)**,** 103-112. doi: 10.1016/j.yhbeh.2013.05.006.

Gray, J.D., Milner, T.A., and McEwen, B.S. (2013). Dynamic plasticity: The role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors *Neuroscience* 239**,** 214-227. doi: 10.1016/j.neuroscience.2012.08.034.

Graziano, M., Benito, R., Planas, J.V., and Palstra, A.P. (2018). Swimming exercise to control precocious maturation in male seabass (*Dicentrarchus labrax*). *BMC Dev. Biol.* 18(1)**,** 10. doi: 10.1186/s12861-018-0170-8.

Harrison, H.L., Kochalski, S., Arlinghaus, R., and Aas, Ø. (2018). “Nature’s Little Helpers”: A benefits approach to voluntary cultivation of hatchery fish to support wild Atlantic salmon (*Salmo salar*) populations in Norway, Wales, and Germany. *Fish. Res.* 204**,** 348-360. doi: 10.1016/j.fishres.2018.02.022.

Hawkins, L.A., Armstrong, J.D., and Magurran, A.E. (2007). A test of how predator conditioning influences survival of hatchery-reared Atlantic salmon, *Salmo salar*, in restocking programmes. *Fish. Manage. Ecol.* 14(4)**,** 291-293. doi: 10.1111/j.1365-2400.2007.00548.x.

Heggenes, J., Baglinière, J.L., and Cunjak, R.A. (1999). Spatial niche variability for young Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in heterogeneous streams. *Ecol. Freshwat. Fish* 8(1)**,** 1-21. doi: 10.1111/j.1600-0633.1999.tb00048.x.

Henderson, J.N., and Letcher, B.H. (2003). Predation on stocked Atlantic salmon (*Salmo salar*) fry. *Can. J. Fish. Aquat. Sci.* 60(1)**,** 32-42. doi: 10.1139/f03-001.

Hodos, W., and Campbell, C.B.G. (1969). Scala naturae: Why there is no theory in comparative psychology. *Psychol. Rev.* 76(4)**,** 337-350. doi: 10.1037/h0027523.

Hoffman, G.E., Smith, M.S., and Verbalis, J.G. (1993). c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Front. Neuroendocrinol.* 14(3)**,** 173-213. doi: 10.1006/frne.1993.1006.

Hoffnagle, T.L., Carmichael, R.W., and Keniry, P.J. (2006). The effect of moderately increased and variable raceway flow rates on juvenile physiology, survival, and adult return of hatchery-reared Chinook salmon. *Trans. Am. Fish. Soc.* 135(6)**,** 1567-1577. doi: 10.1577/T05-246.1.

Huntingford, F., and Kadri, S. (2013). "Exercise, Stress and Welfare" in *Swimming physiology of fish: Towards using exercise to farm a fit fish in sustainable aquaculture,* eds. P.A. Palstra & V.J. Planas. (Berlin, Heidelberg: Springer Berlin Heidelberg), 161-174.

Huntingford, F.A. (2004). Implication of domestication and rearing conditions for the behaviour of cultivated fishes. *J. Fish Biol.* 65**,** 122-142. doi: 10.1111/j.1095-8649.2004.00562.x.

Huntingford, F.A., Metcalfe, N.B., Thorpe, J.E., Graham, W.D., and Adams, C.E. (1990). Social dominance and body size in Atlantic salmon parr, *Salmo salar* L. *J. Fish Biol.* 36(6)**,** 877-881. doi: 10.1111/j.1095-8649.1990.tb05635.x.

Hyvärinen, P., Rodewald, P., and Bradford, M. (2013). Enriched rearing improves survival of hatchery-reared Atlantic salmon smolts during migration in the river Tornionjoki. *Can. J. Fish. Aquat. Sci.* 70(9)**,** 1386-1395. doi: 10.1139/cjfas-2013-0147.

Höglund, E., Sørensen, C., Bakke, M.J., Nilsson, G.E., and Øverli, Ø. (2007). Attenuation of stress-induced anorexia in brown trout (*Salmo trutta*) by pre-treatment with dietary l-tryptophan. *Br. J. Nutr.* 97(4)**,** 786-789. doi: 10.1017/S0007114507450280.

IUCN (2016). *The IUCN red list of threatened species. Version 2016-2* [Online]. Available: http://www.iucnredlist.org [Accessed August 11 2016].

Jin, L., and Lloyd, R.V. (1997). In situ hybridization: Methods and applications. *J. Clin. Lab. Anal.* 11(1)**,** 2-9. doi: 10.1002/(SICI)1098-2825(1997)11:1<2::AID-JCLA2>3.0.CO;2-F.

Johansen, I.B., Sorensen, C., Sandvik, G.K., Nilsson, G.E., Hoglund, E., Bakken, M., et al. (2012). Neural plasticity is affected by stress and heritable variation in stress coping style. *Comp. Biochem. Physiol. Part D* 7(2)**,** 161-171. doi: 10.1016/j.cbd.2012.01.002.

Johansen, M., Erkinaro, J., Amundsen, P.-A., Aas, O., Einum, S., Klemetsen, A., et al. (2011). "The when, what and where of freshwater feeding" in *Atlantic Salmon Ecology,* eds. Ø. Aas, S. Einum, A. Klemetsen & J. Skurdal. Wiley-Blackwell, 89-114.

Johnsen, B.O., Arnekleiv, J.V., Asplin, L., Barlaup, B.T., Næsje, T.F., Rosseland, B.O., et al. (2010). "Hydropower development – ecological effects" in *Atlantic Salmon Ecology,* eds. Ø. Aas, S. Einum, A. Klemetsen & J. Skurdal. Wiley-Blackwell, 351-386.

Johnson, C.N., Balmford, A., Brook, B.W., Buettel, J.C., Galetti, M., Guangchun, L., et al. (2017). Biodiversity losses and conservation responses in the Anthropocene. *Science* 356(6335)**,** 270-275. doi: 10.1126/science.aam9317.

Johnson, J.H., McKeon, J.E., and Dropkin, D.S. (1996). Comparative diets of hatchery and wild Atlantic salmon smolts in the Merrimack river. *N. Am. J. Fish. Manage.* 16(2)**,** 440-444. doi: 10.1577/1548-8675(1996)016<0440:cdohaw>2.3.co;2.

Johnsson, J.I., Brockmark, S., and Näslund, J. (2014). Environmental effects on behavioural development consequences for fitness of captive-reared fishes in the wild. *J. Fish Biol.* 85(6)**,** 1946-1971. doi: 10.1111/jfb.12547.

Jonsson, B., and Jonsson, N. (Year). "Restoration and enhancement of salmonid populations and habitats with special reference to Atlantic salmon", in: *Challenges for Diadromous Fishes in a Dynamic Global Environment, American Fisheries Society Symposium*, eds. A.J. Haro, T.S. Avery & K.L. Beal), 497-535.

Jonsson, B., Jonsson, N., and Hansen, L.P. (1991). Differences in life history and migratory behaviour between wild and hatchery-reared Atlantic salmon in nature. *Aquaculture* 98**,** 69-78. doi: 10.1139/f96-090.

Jonsson, B., and Ruud-Hansen, J. (1985). Water temperature as the primary influence on timing of seaward migrations of Atlantic salmon (*Salmo salar*) smolts. *Can. J. Fish. Aquat. Sci.* 42(3)**,** 593-595. doi: 10.1139/f85-076.

Jonsson, N., Jonsson, B., and Hansen, L.P. (1998). Long-term study of the ecology of wild Atlantic salmon smolts in a small Norwegian river. *J. Fish Biol.* 52**,** 638-650. doi: 10.1111/j.1095-8649.1998.tb02023.x

Jonsson, N., Jonsson, B., and Ruud-Hansen, J. (1988). Downstream displacement and life-history traits of whitefish,*Coregonus lavaretus*, in a Norwegian river. *Environ. Biol. Fishes* 23(3)**,** 197-204. doi: 10.1007/BF00004910.

Jøranlid, A.K. (2017). "The policy relating to hatchery and stocking activities in Norway - managing risks and benefits". NASCO Agenda item 6.1, retrieved on 06.09.2018 from http://www.nasco.int/pdf/2017%20papers/CNL_17_44_AnneKristin.pdf

Kallio-Nyberg, I., Saloniemi, I., Jutila, E., and Jokikokko, E. (2011). Effect of hatchery rearing and environmental factors on the survival, growth and migration of Atlantic salmon in the Baltic Sea. *Fish. Res.* 109(2-3)**,** 285-294. doi: 10.1016/j.fishres.2011.02.015.

Kallio-Nyberg, I., Jutila, E., Saloniemi, I., and Jokikokko, E. (2004). Association between environmental factors, smolt size and the survival of wild and reared Atlantic salmon from the Simojoki River in the Baltic Sea. *J. Fish Biol.* 65(1)**,** 122-134. doi: 10.1111/j.0022-1112.2004.00435.x.

Kalueff, A.V., Stewart, A.M., and Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol. Sci.* 35(2)**,** 63-75. doi: 10.1016/j.tips.2013.12.002.

Kaslin, J., Ganz, J., and Brand, M. (2008). Proliferation, neurogenesis and regeneration in the non-mammalian vertebrate brain. *Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci.* 363(1489)**,** 101-122. doi: 10.1098/rstb.2006.2015.

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., A: Mol. Integr. Physiol.* 145(2)**,** 145-151. doi: 10.1016/j.cbpa.2006.06.041.

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.

Kinoshita, M., Ito, E., Urano, A., Ito, H., and Yamamoto, N. (2006). Periventricular efferent neurons in the optic tectum of rainbow trout. *J. Comp. Neurol.* 499(4)**,** 546-564. doi: 10.1002/cne.21080.

Klovach, N.V., Temnykh, O.S., Shevlyakov, V.A., Golub, E.V., Kanzeporova, A.N., Shevlyakov, E.A., et al. (2015). "Biostatistical information on salmon catches, escapement and enhancement production in Russia in 2014". NPAFC Doc. 1565 (Rev. 2) 4 pp. Available at http://www.npafc.org.

Kotrschal, K., Van Staaden, M., and Huber, R. (1998). Fish brains: Evolution and anvironmental relationships. *Rev. Fish Biol. Fish.* 8(4)**,** 373-408. doi: 10.1023/A:1008839605380.

Lagasse, J.P., Leith, D.A., Romey, D.B., and Dahrens, O.F. (1980). Stamina and survival of Coho salmon reared in rectangular circulating ponds and conventional raceways. *Prog. Fish Cult.* 42(3)**,** 153-156. doi: 10.1577/1548-8659(1980)42[153:SASOCS]2.0.CO;2.

Lambert, T.J., Fernandez, S.M., and Frick, K.M. (2005). Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. *Neurobiol. Learn. Mem.* 83(3)**,** 206-216. doi: 10.1016/j.nlm.2004.12.001.

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.

Lau, B.Y.B., Mathur, P., Gould, G.G., and Guo, S. (2011). Identification of a brain center whose activity discriminates a choice behavior in zebrafish. *Proc. Natl. Acad. Sci.* 108(6)**,** 2581-2586. doi: 10.1073/pnas.1018275108.

Lee, J.S.F., and Berejikian, B.A. (2008). Effects of the rearing environment on average behaviour and behavioural variation in steelhead. *J. Fish Biol.* 72(7)**,** 1736-1749. doi: 10.1111/j.1095-8649.2008.01848.x.

Leon, K., A. (1986). Effect of exercise on feed consumption, growth, food conversion, and stamina of brook trout. *Prog. Fish Cult.* 48(1)**,** 43-46. doi: 10.1577/1548-8640(1986)48<43:EOEOFC>2.0.CO;2.

Lien, S., Koop, B.F., Sandve, S.R., Miller, J.R., Kent, M.P., Nome, T., et al. (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature* 533**,** 200-205. doi: 10.1038/nature17164.

Lieschke, G.J., and Currie, P.D. (2007). Animal models of human disease: zebrafish swim into view. *Nat. Rev. Genet.* 8**,** 353-367. doi: 10.1038/nrg2091.

Lista, I., and Sorrentino, G. (2010). Biological mechanisms of physical activity in preventing cognitive decline. *Cell. Mol. Neurobiol.* 30(4)**,** 493-503. doi: 10.1007/s10571-009-9488-x.

Liu, X., Ramirez, S., Pang, P.T., Puryear, C.B., Govindarajan, A., Deisseroth, K., et al. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* 484**,** 381-385. doi: 10.1038/nature11028.

Luchiari, A.C., and Chacon, D.M.M. (2013). Physical exercise improves learning in zebrafish, *Danio rerio*. *Behav. Process.* 100**,** 44-47. doi: 10.1016/j.beproc.2013.07.020.

Ma, C.-L., Ma, X.-T., Wang, J.-J., Liu, H., Chen, Y.-F., and Yang, Y. (2017). Physical exercise induces hippocampal neurogenesis and prevents cognitive decline. *Behav. Brain Res.* 317**,** 332-339. doi: 10.1016/j.bbr.2016.09.067.

Ma, D.K., Jang, M.-H., Guo, J.U., Kitabatake, Y., Chang, M.-l., Pow-anpongkul, N., et al. (2009). Neuronal activity–induced *Gadd45b* promotes epigenetic DNA demethylation and adult neurogenesis. *Science* 323(5917)**,** 1074-1077. doi: 10.1126/science.1166859.

Maier, T., Güell, M., and Serrano, L. (2009). Correlation of mRNA and protein in complex biological samples. *FEBS Lett.* 583(24)**,** 3966-3973. doi: 10.1016/j.febslet.2009.10.036.

Manuel, R., Gorissen, M., Stokkermans, M., Zethof, J., Ebbesson, L.O.E., Vis, H.v.d., et al. (2015). The effects of environmental enrichment and age-related differences on inhibitory avoidance in zebrafish (*Danio rerio* Hamilton). *Zebrafish* 12(2)**,** 152-165. doi: 10.1089/zeb.2014.1045.

Marchetti, M.P., and Nevitt, G.A. (2003). Effects of hatchery rearing on brain structures of rainbow trout, *Oncorhynchus mykiss*. *Environ. Biol. Fishes* 66**,** 9-14. doi: 10.1023/A:1023269221678.

Martin, R., M., and Wertheimer, A. (1989). Adult production of Chinook salmon reared at different densities and released as two smolt sizes. *Prog. Fish Cult.* 51(4)**,** 194-200. doi: 10.1577/1548-8640(1989)051<0194:APOCSR>2.3.CO;2.

Mattson, M.P., Maudsley, S., and Martin, B. (2004). BDNF and 5-HT: A dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* 27(10)**,** 589-594. doi: 10.1016/j.tins.2004.08.001.

Maynard, D.J., Flagg, T.A., and Mahnken, C.V.W. (1995). "A review of seminatural culture strategies for enhancing the postrelease survival of anadromous salmonids", in: *Am. Fish. Soc. Symp.* 15, 307-314.26

Maynard, D.J., and Trial, J.G. (2013). The use of hatchery technology for the conservation of Pacific and Atlantic salmon. *Rev. Fish Biol. Fish.* 24(3), 803-817. doi: 10.1007/s11160-013-9341-7.

McCormick, S.D., Hansen, L.P., Quinn, T.P., and Saunders, R.L. (1998). Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 55(S1), 77-92. doi: 10.1139/d98-011.

McEwen, B.S., and Wingfield, J.C. (2003). The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43(1), 2-15. doi: 10.1016/S0018-506X(02)00024-7.

Mes, D., Dirks, R.P., and Palstra, A.P. (2016). Simulated migration under mimicked photothermal conditions enhances sexual maturation of farmed European eel (*Anguilla anguilla*). *Aquaculture* 452, 367-372. doi: 10.1016/j.aquaculture.2015.11.020.

Metcalfe, N.B. (1986). Intraspecific variation in competitive ability and food intake in salmonids: Consequences for energy budgets and growth rates. *J. Fish Biol.* 28(5), 525-531. doi: 10.1111/j.1095-8649.1986.tb05190.x.

Metcalfe, N.B., Huntingford, F.A., Graham, W.D., and Thorpe, J.E. (1989). Early social status and the development of life-history strategies in Atlantic salmon. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* 236(1282), 7-19. doi: 10.1098/rspb.1989.0009.

Metzker, M.L. (2009). Sequencing technologies — the next generation. *Nat. Rev. Genet.* 11, 31-46. doi: 10.1038/nrg2626.

Millidine, K.J., Armstrong, J.D., and Metcalfe, N.B. (2006). Presence of shelter reduces maintenance metabolism of juvenile salmon. *Funct. Ecol.* 20(5), 839-845. doi: 10.1111/j.1365-2435.2006.01166.x.

Molteni, R., Ying, Z., and Gómez-Pinilla, F. (2002). Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. *Eur. J. Neurosci.* 16(6), 1107-1116. doi: 10.1046/j.1460-9568.2002.02158.x.

Morgane, P.J., Galler, J.R., and Mokler, D.J. (2005). A review of systems and networks of the limbic forebrain/limbic midbrain. *Prog. Neurobiol.* 75(2), 143-160. doi: 10.1016/j.pneurobio.2005.01.001.

Munakata, A., Björnsson, B.T., Jönsson, E., Amano, M., Ikuta, K., Kitamura, S., et al. (2000). Post-release adaptation processes of hatchery-reared honmasu salmon parr. *J. Fish Biol.* 56, 163-172. doi: 10.1111/j.1095-8649.2000.tb02092.x.

Nakashima, A., and Sasaki, K. (2014). "Preliminary 2013 salmon enhancement production in Japan". NPAFC Doc. 1516, 3pp. Available at www.npafc.org.

Newman, M., Ebrahimie, E., and Lardelli, M. (2014). Using the zebrafish model for Alzheimer's disease research. *Front. Genet.* 5, 189. doi: 10.3389/fgene.2014.00189.

Noble, C., Kadri, S., Mitchell David, F., and Huntingford Felicity, A. (2007). Influence of feeding regime on intraspecific competition, fin damage and growth in 1+ Atlantic salmon parr (*Salmo salar* L.) held in freshwater production cages. *Aquacult. Res.* 38(11), 1137-1143. doi: 10.1111/j.1365-2109.2007.01777.x.

Northcutt, R.G. (2006). Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *J. Comp. Neurol.* 494(6), 903-943. doi: 10.1002/cne.20853.

Näslund, J., Aarestrup, K., Thomassen, Søren T., Johnsson, Jörgen I., and Post, J. (2012). Early enrichment effects on brain development in hatchery-reared Atlantic salmon (*Salmo salar*): no evidence for a critical period. *Can. J. Fish. Aquat. Sci.* 69(9), 1481-1490. doi: 10.1139/f2012-074.

Näslund, J., and Johnsson, J.I. (2014). Environmental enrichment for fish in captive environments: effects of physical structures and substrates. *Fish Fish.* 17(1), 1-30. doi: 10.1111/faf.12088.

Näslund, J., Rosengren, M., Del Villar, D., Gansel, L., Norrgård, J.R., Persson, L., et al. (2013). Hatchery tank enrichment affects cortisol levels and shelter-seeking in Atlantic

salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 70(4), 585-590. doi: 10.1139/cjfas-2012-0302.

O'Connell, L.A., and Hofmann, H.A. (2011). The vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *J. Comp. Neurol.* 519(18), 3599-3639. doi: 10.1002/cne.22735.

Okuno, H. (2011). Regulation and function of immediate-early genes in the brain: Beyond neuronal activity markers. *Neurosci. Res.* 69(3), 175-186. doi: 10.1016/j.neures.2010.12.007.

Oliveira, R.F. (2009). Social behavior in context: Hormonal modulation of behavioral plasticity and social competence. *Integr. Comp. Biol.* 49(4), 423-440. doi: 10.1093/icb/icp055.

Olla, B.L., Davis, M.W., and Ryer, C.H. (1998). Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bull. Mar. Sci.* 62(2), 531-550.

Palmé, A., Wennerstöm, L., Guban, P., Ryman, N., and Laikre, L. (2012). Compromising Baltic salmon genetic diversity - conservation genetic risks assiciated with compensatory releases of salmon in the Balctic Sea. *Havs- och vattenmyndighetens rapport 2012:18*, 115 pp.

Palstra, A.P., Crespo, D., van den Thillart, G.E.E.J.M., and Planas, J.V. (2010). Saving energy to fuel exercise: Swimming suppresses oocyte development and downregulates ovarian transcriptomic response of rainbow trout *Oncorhynchus mykiss*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299(2), R486-R499. doi: 10.1152/ajpregu.00109.2010.

Palstra, A.P., Mes, D., Kusters, K., Roques, J.A., Flik, G., Kloet, K., et al. (2015). Forced sustained swimming exercise at optimal speed enhances growth of juvenile yellowtail kingfish (*Seriola lalandi*). *Front. Physiol.* 5, 506. doi: 10.3389/fphys.2014.00506.

Pang, P.T., Teng, H.K., Zaitsev, E., Woo, N.T., Sakata, K., Zhen, S., et al. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* 306(5695), 487-491. doi: 10.1126/science.1100135.

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, 281-287. doi: 10.1139/cjfas-55-S1-281.

Pavlidis, M., Theodoridi, A., and Tsalafouta, A. (2015). Neuroendocrine regulation of the stress response in adult zebrafish, *Danio rerio*. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 60, 121-131. doi: 10.1016/j.pnpbp.2015.02.014.

Pfaffl, M.W., Tichopad, A., Prgomet, C., and Neuvians, T.P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations. *Biotechnol. Lett.* 26(6), 509-515. doi: 10.1023/B:BILE.0000019559.84305.47.

Pickering, A.D., Pottinger, T.G., and Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: A time-course study. *J. Fish Biol.* 20(2), 229-244. doi: 10.1111/j.1095-8649.1982.tb03923.x.

Portavella, M., Torres, B., and Salas, C. (2004). Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. *J. Neurosci.* 24(9), 2335-2342. doi: 10.1523/jneurosci.4930-03.2004.

Pottinger, T.G., Pickering, A.D., and Hurley, M.A. (1992). Consistency in the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 103(3), 275-289. doi: 10.1016/0044-8486(92)90172-H.

Rink, E., and Wullimann, M.F. (2004). Connections of the ventral telencephalon (subpallium) in the zebrafish (*Danio rerio*). *Brain Res.* 1011(2), 206-220. doi: 10.1016/j.brainres.2004.03.027.

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.

Roberts, L.J., Taylor, J., Gough, P.J., Forman, D.W., and Garcia de Leaniz, C. (2014). Silver spoons in the rough: can environmental enrichment improve survival of hatchery Atlantic salmon *Salmo salar* in the wild? *J. Fish Biol.* 85(6), 1972-1991. doi: 10.1111/jfb.12544.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1), 139-140. doi: 10.1093/bioinformatics/btp616.

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. Freshwat. Fish* 20(4), 569-579. doi: 10.1111/j.1600-0633.2011.00505.x.

Rodríguez, F., Broglio, C., Durán, E., Gómez, A., and Salas, C. (2007). "Neural mechanisms of learning in teleost fish" in *Fish Cognition and Behavior,* eds. C. Brown, K. Laland & J. Krause. Blackwell Publishing.

Rosengren, M., Kvingedal, E., Näslund, J., Johnsson, J.I., and Sundell, K. (2016). Born to be wild: effects of rearing density and environmental enrichment on stress, welfare, and smolt migration in hatchery-reared Atlantic salmon. *Can. J. Fish. Aquat. Sci.* 74(3), 1-10. doi: 10.1139/cjfas-2015-0515.

Rosseland, B.O., and Kroglund, F. (2010). "Lessons from acidification and pesticides" in *Atlantic Salmon Ecology,* eds. Ø. Aas, S. Einum, A. Klemetsen & J. Skurdal. Wiley-Blackwell, 387-408.

Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T., et al. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 18(1), 529. doi: 10.1186/s12859-017-1934-z.

Salas, C., Broglio, C., Durán, E., Gómez, A., Ocaña, F.M., Jiménez-Moya, F., et al. (2006). Neuropsychology of learning and memory in teleost fish. *Zebrafish* 3(2), 157-171. doi: 10.1089/zeb.2006.3.157.

Salvanes, A.G., Moberg, O., Ebbesson, L.O., Nilsen, T.O., Jensen, K.H., and Braithwaite, V.A. (2013). Environmental enrichment promotes neural plasticity and cognitive ability in fish. *Proc. R. Soc. B* 280(1767), 20131331. doi: 10.1098/rspb.2013.1331.

Salvanes, A.G.V. (2017). Are antipredator behaviours of hatchery *Salmo salar* juveniles similar to wild juveniles? *J. Fish Biol.* 90(5), 1785-1796. doi: 10.1111/jfb.13268.

Santana, S., Rico, E.P., and Burgos, J.S. (2012). Can zebrafish be used as animal model to study Alzheimer's disease? *Am. J. Neurodegener. Dis.* 1(1), 32-48.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676-682. doi: 10.1038/nmeth.2019.

Shors, T.J., Anderson, M.L., Curlik, D.M., and Nokia, M.S. (2012). Use it or lose it: How neurogenesis keeps the brain fit for learning. *Behav. Brain Res.* 227(2), 450-458. doi: 10.1016/j.bbr.2011.04.023.

Silver, N., Best, S., Jiang, J., and Thein, S.L. (2006). Selection of housekeeping genes for gene expression studies in human reticulocytes using real-time PCR. *BMC Mol. Biol.* 7, 33. doi: 10.1186/1471-2199-7-33.

Singewald, G.M., Rjabokon, A., Singewald, N., and Ebner, K. (2011). The modulatory role of the lateral septum on neuroendocrine and behavioral stress responses. *Neuropsychopharmacology* 36(4), 793-804. doi: 10.1038/npp.2010.213.

Skilbrei, O.T., and Holm, M. (1998). Effects of long-term exercise on survival, homing and straying of released Atlantic salmon smolts. *J. Fish Biol.* 52(5)**,** 1083-1086. doi: 10.1111/j.1095-8649.1998.tb00606.x.

Skilbrei, O.T., Holm, M., JØRstad, K.E., and Handeland, S.A. (1994). Migration motivation of cultured Atlantic salmon, *Salmo salar* L., smolts in relation to size, time of release and acclimatization period. *Aquacult. Res.* 25(S2)**,** 65-77. doi: 10.1111/are.1994.25.s2.65.

Summers, C.H., and Winberg, S. (2006). Interactions between the neural regulation of stress and aggression. *J. Exp. Biol.* 209(23)**,** 4581-4589. doi: 10.1242/jeb.02565.

Sundström, L.F., and Johnsson, J.I. (2001). Experience and social environment influence the ability of young brown trout to forage on live novel prey. *Anim. Behav.* 61(1)**,** 249-255. doi: 10.1006/anbe.2000.1593.

Symons, P.E.K., and Heland, M. (1978). Stream habitats and behavioral interactions of underyearling and yearling Atlantic salmon (*Salmo salar*). *J. Fish. Res. Board Can.* 35(2)**,** 175-183. doi: 10.1139/f78-029.

Sørensen, C., Bohlin, L.C., Øverli, Ø., and Nilsson, G.E. (2011). Cortisol reduces cell proliferation in the telencephalon of rainbow trout (*Oncorhynchus mykiss*). *Physiol. Behav.* 102(5)**,** 518-523. doi: 10.1016/j.physbeh.2010.12.023.

Tatara, C.P., Riley, S.C., and Scheurer, J.A. (2009). Growth, survival, and habitat use of naturally reared and hatchery steelhead fry in streams: Effects of an enriched hatchery rearing environment. *Trans. Am. Fish. Soc.* 138(3)**,** 441-457. doi: 10.1577/t07-260.1.

Taupin, P. (2007). BrdU immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation. *Brain Res. Rev.* 53(1)**,** 198-214. doi: 10.1016/j.brainresrev.2006.08.002.

Teixeira, A., and Cortes, R.M.V. (2006). Diet of stocked and wild trout, *Salmo trutta*: Is there competition for resources? *Folia Zool.* 55(1)**,** 61-73.

Thorstad, E., B., Whoriskey, F., Rikardsen Audun, H., and Aarestrup, K. (2010). "Aquatic nomads: The life and migrations of the Atlantic salmon" in *Atlantic Salmon Ecology,* eds. Ø. Aas, S. Einum, A. Klemetsen & J. Skurdal. Wiley-Blackwell, 1-32.

Thorstad, E.B., Fiske, P., and Forseth, T. (2017). Klassifisering av 148 laksebestander etter kvalitetsnorm for villaks. *Temarapport fra vitenskapelig råd for lakseforfaltning* (Nr. 5)**,** 81 pp.

Thorstad, E.B., and Forseth, T. (2017). "Status for norske laksebestander i 2017", in: *Vitenskapelig Råd for Lakseforvaltning*. Report nr. 10, 152 pp.

Thorstad, E.B., Uglem, I., Arechavala-Lopez, P., Økland, F., and Finstad, B. (2011). Low survival of hatchery-released Atlantic salmon smolts during initial river and fjord migration. *Boreal Environ. Res.* 16**,** 115-120.

Thorstad, E.B., Whoriskey, F., Uglem, I., Moore, A., Rikardsen, A.H., and Finstad, B. (2012). A critical life stage of the Atlantic salmon *Salmo salar*: Behaviour and survival during the smolt and initial post-smolt migration. *J. Fish Biol.* 81(2)**,** 500-542. doi: 10.1111/j.1095-8649.2012.03370.x.

Tognoli, C., Rossi, F., Di Cola, F., Baj, G., Tongiorgi, E., Terova, G., et al. (2010). Acute stress alters transcript expression pattern and reduces processing of proBDNF to mature BDNF in *Dicentrarchus labrax*. *BMC Neurosci.* 11(1)**,** 4. doi: 10.1186/1471-2202-11-4.

Tompkins, A., Hamilton, S., and Bateman, J. (2015). "Canadian salmon catch and enanced salmon production in 2013 and 2014". NPAFC Doc. 1606. 7 pp. Fisheries and Oceans Canada. Available at http://www.npafc.org.

Tudorache, C., de Boeck, G., and Claireaux, G. (2013). "Forced and preferred swimming speeds of fish: A methodological approach" in *Swimming Physiology of Fish: Towards Using Exercise to Farm a Fit Fish in Sustainable Aquaculture,* eds. A.P. Palstra & J.V. Planas. (Berlin, Heidelberg: Springer Berlin Heidelberg), 81-108.

Tudorache, C., O'Keefe, R.A., and Benfey, T.J. (2011). Optimal swimming speeds reflect preferred swimming speeds of brook charr (*Salvelinus fontinalis* Mitchill, 1874). *Fish Physiol. Biochem.* 37(2)**,** 307-315. doi: 10.1007/s10695-011-9498-8.

van der Borght, K., Havekes, R., Bos, T., Eggen, B.J., and van der Zee, E.A. (2007). Exercise improves memory acquisition and retrieval in the Y-maze task: Relationship with hippocampal neurogenesis. *Behav. Neurosci.* 121(2)**,** 324-334. doi: 10.1037/0735-7044.121.2.324.

van Praag, H. (2008). Neurogenesis and exercise: Past and future directions. *Neuromolecular Med.* 10(2)**,** 128-140. doi: 10.1007/s12017-008-8028-z.

van Praag, H., Christie, B.R., Sejnowski, T.J., and Gage, F.H. (1999a). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc. Natl. Acad. Sci.* 96(23)**,** 13427-13431. doi: 10.1073/pnas.96.23.13427.

van Praag, H., Kempermann, G., and Gage, F.H. (1999b). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* 2(3)**,** 266-270. doi: 10.1038/6368.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 3(7)**,** 1-12. doi: 10.1186/gb-2002-3-7-research0034.

Vargas, J.P., López, J.C., and Portavella, M. (2009). What are the functions of fish brain pallium? *Brain Res. Bull.* 79(6)**,** 436-440. doi: 10.1016/j.brainresbull.2009.05.008.

Vaynman, S., Ying, Z., and Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur. J. Neurosci.* 20(10)**,** 2580-2590. doi: 10.1111/j.1460-9568.2004.03720.x.

Vercessi, L. (2015). "Alaska salmon fisheries enhancement program 2014 annual report". Alaska Department of Fish and Game, Fishery management report No. 15-15, Anchorage, USA.

Vindas, M.A., Fokos, S., Pavlidis, M., Höglund, E., Dionysopoulou, S., Ebbesson, L.O.E., et al. (2018). Early life stress induces long-term changes in limbic areas of a teleost fish: The role of catecholamine systems in stress coping. *Sci. Rep.* 8(1)**,** 5638. doi: 10.1038/s41598-018-23950-x.

Vindas, M.A., Gorissen, M., Höglund, E., Flik, G., Tronci, V., Damsgård, B., et al. (2017). How do individuals cope with stress? Behavioural, physiological and neuronal differences between proactive and reactive coping styles in fish. *J. Exp. Biol.***,** 1524-1532. doi: 10.1242/jeb.153213.

Vivar, C., Potter, M.C., and van Praag, H. (2013). "All about running: Synaptic plasticity, growth factors and adult hippocampal neurogenesis" in *Neurogenesis and Neural Plasticity,* eds. C. Belzung & P. Wigmore. (Berlin, Heidelberg: Springer Berlin Heidelberg), 189-210.

von Bohlen und Halbach, O. (2007). Immunohistological markers for staging neurogenesis in adult hippocampus. *Cell Tissue Res.* 329(3)**,** 409-420. doi: 10.1007/s00441-007-0432-4.

von Krogh, K., Sorensen, C., Nilsson, G.E., and Overli, O. (2010). Forebrain cell proliferation, behavior, and physiology of zebrafish, *Danio rerio*, kept in enriched or barren environments. *Physiol. Behav.* 101(1)**,** 32-39. doi: 10.1016/j.physbeh.2010.04.003.

Voss, M.W., Vivar, C., Kramer, A.F., and van Praag, H. (2013). Bridging animal and human models of exercise-induced brain plasticity. *Trends Cogn. Sci.* 17(10)**,** 525-544. doi: 10.1016/j.tics.2013.08.001.

Wendelaar Bonga, S.E. (1997). The stress response in fish. *Physiol. Rev.* 77(3)**,** 591-625. doi: 10.1152/physrev.1997.77.3.591.

Williams, S.N., and Undieh, A.S. (2009). Dopamine D1-like receptor activation induces brain-derived neurotrophic factor protein expression. *Neuroreport* 20(6)**,** 606-610. doi: 10.1097/WNR.0b013e32832a0a98.

Winberg, S., and Nilsson, G.E. (1993). Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. *Comp. Biochem. Physiol., C: Pharmacol. Toxicol. Endocrinol.* 106(3)**,** 597-614. doi: 10.1016/0742-8413(93)90216-8.

Wood, L.S., Desjardins, J.K., and Fernald, R.D. (2011). Effects of stress and motivation on performing a spatial task. *Neurobiol. Learn. Mem.* 95(3)**,** 277-285. doi: 10.1016/j.nlm.2010.12.002.

Wullimann, M.F., Rupp, B., and Reichert, H. (1996). "The brain of the zebrafish *Danio rerio*: a neuroanatomical atlas" in *Neuroanatomy of the Zebrafish Brain: A Topological Atlas,* eds. M.F. Wullimann, B. Rupp & H. Reichert. (Basel: Birkhäuser Basel), 19-87.

Young, M.D., Wakefield, M.J., Smyth, G.K., and Oshlack, A. (2010). Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biol.* 11(2)**,** R14. doi: 10.1186/gb-2010-11-2-r14.

Zohar, Y., Muñoz-Cueto, J.A., Elizur, A., and Kah, O. (2010). Neuroendocrinology of reproduction in teleost fish. *Gen. Comp. Endocrinol.* 165(3)**,** 438-455. doi: 10.1016/j.ygcen.2009.04.017.

Zupanc, G.K.H. (2006). Neurogenesis and neuronal regeneration in the adult fish brain. *J. Comp. Physiol., A* 192(6)**,** 649-670. doi: 10.1007/s00359-006-0104-y.

Zupanc, G.K.H. (2008). Adult neurogenesis and neuronal regeneration in the brain of teleost fish. *J. Physiol. Paris* 102(4-6)**,** 357-373. doi: 10.1016/j.jphysparis.2008.10.007.

Zupanc, G.K.H., and Sîrbulescu, R.F. (2011). Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. *Eur. J. Neurosci.* 34(6)**,** 917-929. doi: 10.1111/j.1460-9568.2011.07854.x.

Øverli, Ø., Winberg, S., Damsård, B., and Jobling, M. (1998). Food intake and spontaneous swimming activity in Arctic char (*Salvelinus alpinus*): Role of brain serotonergic activity and social interactions. *Can. J. Zool.* 76(7)**,** 1366-1370. doi: 10.1139/z98-050.

Appendix: Papers I-III

I

"Rivers and the inhabitants of the watery element were made for wise men to contemplate, and fools to pass by without consideration."

Izaak Walton (1593-1683)

Neurobiology of wild and hatchery-reared Atlantic salmon: how nurture drives neuroplasticity

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Abstract

Life experiences in the rearing environment shape the neural and behavioral plasticity of animals. In fish stocking practices, the hatchery environment is relatively stimulus-deprived and does not optimally prepare fish for release into the wild. While the behavioral differences between wild and hatchery-reared fish have been examined to some extent, few studies have compared neurobiological characteristics between wild and hatchery-reared individuals. Here, we compare the expression of immediate early gene *cfos* and neuroplasticity marker *brain derived neurotrophic factor* (*bdnf*) in telencephalic subregions associated with processing of stimuli in wild and hatchery-reared Atlantic salmon at basal and 30 min post (acute) stress conditions. Using *in situ* hybridization, we found that the expression level of these markers is highly specific per neuronal region and affected by both the origin of the fish, and exposure to acute stress. Expression of *cfos* was increased by stress in all brain regions and *cfos* was more highly expressed in the Dlv (functional equivalent to the mammalian hippocampus) of hatchery-reared compared to wild fish. Expression of *bdnf* was higher overall in hatchery fish, while acute stress upregulated *bdnf* in the Dm (functional equivalent to the mammalian amygdala) of wild, but not hatchery individuals. Our findings demonstrate that the hatchery environment affects neuroplasticity and neural activation in brain regions that are important for learning processes and stress reactivity, providing a neuronal foundation for the behavioral differences observed between wild and hatchery-reared fish.

Keywords: *cfos*, *bdnf*, Atlantic salmon, immediate early gene, fish stocking, neuroplasticity, *in situ* hybridization

1 Introduction

Wild Atlantic salmon (*Salmo salar*, L.) populations are declining worldwide (Parrish et al., 1998). Even in Norway – traditionally home to some of the healthiest Atlantic salmon stocks in the world – the number of wild salmon has more than halved in the last three decades (Thorstad and Forseth, 2015). Habitat degradation is one of the main reasons for salmon decline, and habitat restoration should thus be considered first and foremost as conservation tool (Araki and Schmid, 2010). However, since habitat restoration is a slow and costly process, more immediate measures to support declining population numbers are frequently employed, such as the annual release of millions of hatchery-reared salmon into rivers worldwide through stocking programs (e.g. Palmé et al., 2012; Maynard and Trial, 2013). To this end, mature local salmon are captured and cross-fertilized, after which their offspring are reared in captivity and released in the wild at different developmental stages, ranging from eggs to juveniles but mostly at the smolt stage (Jonsson and Jonsson, 2009; Maynard and Trial, 2013). The hatchery environment provides optimal conditions for growth, which consequently leads to higher growth rates and larger body size at time of release for hatchery-reared fish compared to wild fish of the same age (Jonsson and Jonsson, 2009). However, cultured fish are generally reared under unnaturally high densities in stimulus-poor conditions, which leads to diminished behavioral plasticity in critical life skills such as antipredator and foraging behavior (Olla et al., 1998; Huntingford, 2004; Jonsson and Jonsson, 2009). For example, after release in the wild, stocked salmon often show reduced stomach fullness (Johnson et al., 1996) or ingestion of indigestible particles such as small rocks and plant material (Munakata et al., 2000). Behavioral deficits such as these contribute to lower post-release survival rates of stocked fish compared to their wild conspecifics (Johnson et al., 1996; Jonsson and Jonsson, 2009; Thorstad et al., 2011), raising both financial and ethical concerns for current stocking practices.

To increase the efficacy of stocking programs, research efforts are directed towards improving behavioral responses to stimuli from the natural environment and, ultimately, the fitness of hatchery-reared fish, through implementation of hatchery innovations such as environmental enrichment (rewied by Johnsson et al., 2014), predator conditioning (reviewed by Brown et al., 2013) or foraging training (reviewed by Olla et al., 1998). In order to rear more 'wild-like' fish under hatchery conditions, it is important to first understand how the neurobiological, physiological and behavioral characteristics of hatchery-reared fish differ from those of their wild conspecifics. While behavioral differences between wild and hatchery-reared fish have been described in several studies (e.g. Olla et al., 1998; Huntingford, 2004), the brain – the organ that underlies these behavioral differences – has remained much understudied. Environmental stimuli trigger and reinforce neuronal circuits through mobilization of neuropeptides such as brain-derived neurotrophic factor (Bdnf), which promotes neurogenesis, cell survival and synaptic plasticity, thus altering the wiring of the brain in response to the rearing environment (Mattson et al., 2004; Ebbesson and Braithwaite, 2012; Shors et al., 2012; Gray et al., 2013). This process of brain modification due to environmental inputs is known as neuroplasticity, and reinforcement of neuronal circuits in response to experiences from the rearing environment affects how these neuronal circuits are activated by future stimuli, thus driving the fish's behavior (Ebbesson and Braithwaite, 2012; Shors et al., 2012). Activation of neuronal circuits can be mapped through visualization of immediate early genes (IEGs) such as *cfos*, which is highly expressed after a neuron is activated, enabling us to take a snapshot of neuronal activation patterns in response to a stimulus such as acute stress (Okuno, 2011; Pavlidis et al., 2015). Thus, *cfos* and *bdnf* transcripts are established markers for neural activity and neuroplasticity, respectively, and

they are important tools to help understand how the rearing environment affects the neurobiology of animals.

In vertebrates, cognitive processing is mainly under forebrain control. Therefore, it is imperative that we obtain a better understanding of how the rearing environment shapes forebrain functionality in order to improve fish quality in stocking programs. In contrast to mammals, teleost fish do not possess a cerebral cortex. However, fish telencephalic areas have been found to be functionally equivalent to mammalian forebrain regions and fish are capable of displaying complex behaviors including social decision making and associative learning, which are under forebrain regulation (Vargas et al., 2009; Kalueff et al., 2012; Stewart and Kalueff, 2012; Bshary and Brown, 2014). Within the telencephalon, the dorsolateral (Dl) and dorsomedial (Dm) pallium have been identified as functional equivalents to the mammalian hippocampus and amygdala, respectively (Portavella et al., 2004; O'Connell and Hofmann, 2011; Broglio et al., 2015). The Dl and hippocampus play a role in relational memory of the environment and experiences, while the Dm and amygdala are involved in emotional learning and stress reactivity (Portavella et al., 2004; Vargas et al., 2009; O'Connell and Hofmann, 2011). Importantly, these proposed functional equivalences may in fact not be specific enough, since recent studies have suggested that the Dl and Dm are each composed of dorsal (Dld, Dmd) and ventral (Dlv, Dmv) neuronal subpopulations, each with distinct topology, connectivity patterns and, most likely, functionality (Broglio et al., 2015, Broglio, pers. comm.). The ventral part of the ventral telencephalon (Vv) has been suggested as the putative functional equivalent to the mammalian lateral septum (LS), which mediates social behavior and regulates goal-oriented behavior (O'Connell and Hofmann, 2011; Singewald et al., 2011). Together, the Dl, Dm and Vv subregions (Fig. 1) of the telencephalon are thus drivers of cognitive processes that are important for behavioral adaptation to novel environments.

To date, the few studies that compare the neurobiology of hatchery-reared fish to that of wild conspecifics have assessed neuroplasticity markers either at the level of the whole brain (Aubin-Horth et al., 2005) or large brain structures such as the hind- and mid-brain (Dunlap et al., 2011). While these studies indicated that the hatchery environment affects neuronal cell proliferation and gene expression patterns, it remains challenging to interpret how these neurobiological differences may be linked to behavior because, to our knowledge, no study has ever compared neuroplasticity markers between wild and hatchery-reared fish on the scale of specific neuronal populations. We sampled wild and hatchery-reared Atlantic salmon parr (juvenile freshwater fish) under basal and acute stress conditions in order to characterize their neurobiology in terms of *cfos* and *bdnf* transcript abundance in the Dld, Dlv, Dmd, Dmv and Vv subregions of the telencephalon. We hypothesize that the rearing environment affects the expression of brain plasticity markers in these subregions, which are important for learning, memory and stress reactivity, and that this may, in part, explain the reported behavioral differences observed between wild and hatchery-reared salmonids. Here we present, for the first time, a detailed study that highlights differences in region-specific telencephalic gene expression between wild and hatchery-reared Atlantic salmon.

2 Material and Methods

2.1 Ethics statement

This experiment was performed under current Norwegian law for experimentation and procedures on live animals and was approved by the Norwegian Food Safety Authority (Mattilsynet) through FOTS application ID 10494.

2.2 Animal origin and conditions

Hatchery-reared and wild Atlantic salmon parr were sampled at the Norwegian Institute for Nature Research (NINA) research station at Ims, Norway, and from the adjacent river Imsa on September 12-13, 2016. The hatchery-reared fish were first generation offspring from wild parents from the river Imsa and thus of the same genetic origin as the sampled wild fish. These wild parents were captured from the river Imsa in November 2015, eggs and milt were harvested and cross-fertilized and the eggs hatched in late January 2016. Larvae started feeding mid-March in 4 m^3 indoor tanks. Fish were transferred to a 50 m^3 indoor concrete rearing tank at five months of age in June 2016. Approximately 300 of these parr were transferred to a 4 m^3 holding tank one month prior to this experiment, where they were housed until sampling. Hatchery fish received flow-through natural water from the river Imsa, mixed with salt water to achieve a final salinity between 1 - 2.5‰. Rearing salmon parr in very dilute saline water is a standard hatchery procedure due to its known health benefits, particularly in combatting freshwater fungi (Long et al., 1977). Juvenile fish were fed commercial feed (Nutra Parr, Skretting, Stavanger, Norway) throughout the day by an automatic feeder.

2.3 Experimental setup and sampling

Wild and hatchery-reared salmon were collected at either basal or acute post-stress conditions (n = 7 per group; 28 fish in total). Both juvenile males and females were sampled and premature males were excluded: sex and premature maturation was verified by dissection after sampling. The sex ratios (M:F) for each group were as follows: hatchery basal: 5:2, hatchery stressed: 2:5, wild basal: 3:4, wild stressed: 2:5. Wild parr were sampled on September 13 from the river Imsa, approximately 500 m upstream from the estuary (58.901385, 5.957336), by electrofishing (Geomega type FA-4, Terik Technology, Levanger, Norway; 700 V). Electrofishing at this voltage does not kill the fish but merely stuns them for a few seconds, allowing enough time for capture. During electrofishing, we worked our way upstream to reduce the risk of catching fish that had previously been stunned and flushed downstream by the water current. Because wild fish were captured by electrofishing, we also subjected all hatchery-reared fish to a similar electric shock to reduce handling differences between treatments. To this end, prior to being sampled, hatchery-reared fish were individually collected by net from the 4 m^3 holding tank and immediately transferred into a 150 L tank where they were stunned for 2 seconds with the same electrofishing equipment. All hatchery fish were sampled from the same tank on September 12. In order to minimize stress, repeated netting was avoided and the fish were processed immediately after netting. We verified that this capture procedure did not cause accumulative stress in the salmon in the holding tank from the observation that plasma cortisol levels of hatchery-reared fish did not increase throughout the sampling process, as the day progressed. In order to sample fish (both wild and hatchery) at basal conditions, individuals were anesthetized immediately after the electroshock in 0.75‰ (v/v) 2-phenoxyethanol (Sigma-Aldrich 77699), which rendered them unconscious within 30 seconds, at which point fish were quickly processed (see below). In order to sample fish post-stress, fish were subjected to a confinement stress, which is a commonly used paradigm which subjects fish to a standardized stressor (e.g. Pottinger et al., 1992). Individuals were subjected to a 30 min confinement stress by placing them in isolation in a 10 L bucket filled with 2 L of river water (bottom diameter: 200 mm, water depth: 65 mm). This confinement bucket was covered with a polystyrene lid and air was constantly supplied by a pump and a submersed aeration stone. The confinement bucket was rinsed thoroughly after every fish to remove any type of biological products that may have been excreted by previous fish. After confinement, stressed fish were anesthetized in the same way

as described above for individuals collected at basal conditions and subsequently processed as described below. Fish at basal and stress conditions were sampled alternatingly to avoid time-of-day effects. After anesthetization, all fish were processed immediately: body mass and fork length were recorded and a blood sample was extracted from the caudal vein using cold heparinized syringes fitted with a 23G needle. Blood samples were kept on ice during sampling, followed by centrifugation for 5 min at 2,300 × *g* to collect plasma, which was subsequently stored at -20°C for two days and then at -80°C until cortisol analysis. Fish were fixed by vascular perfusion with freshly made ice-cold 2% paraformaldehyde (PFA; Sigma-Aldrich P6148) in 0.1 M Sørensen's phosphate buffer (PB; 28 mM NaH_2PO_4, 71 mM Na_2HPO_4, pH 7.2). Brains were then dissected out within two min and post-fixed overnight in 2% PFA in PB at 4˚C. Brain tissue was washed three times for 20 min in Sørensen's PB at room temperature and cryopreserved overnight in 25% sucrose (Sigma-Aldrich S9378) in Sørensen's PB at 4°C. Tissues were then embedded in Tissue-Tek OCT compound (Sakura Finetek) in custom-made silicon molds, frozen on dry ice, wrapped in parafilm and stored at -80°C in 50 ml falcon tubes that contained 5 ml of frozen Milli-Q water to prevent dehydration.

2.4 Cortisol analysis

Plasma cortisol concentrations were determined by radioimmunoassay according to Gorissen et al. (2012). The primary antibody shows a 100% cross reactivity with cortisol, 0.9% with 11-deoxycortisol, 0.6% with cortiscosterone, and < 0.01% with 11-deoxycorticosterone, progesterone, 17-hydroxyprogesterone, testosterone and estradiol. All wells except the 'non-specifics' received 100 µl cortisol antibody (cortisol antibody [SM210], monoclonal and IgG purified; Abcam Cat# ab1949, RRID:AB_302703); 1:2000 and were incubated overnight at 4°C. The following day, the plates were washed three times with 200 µl/well wash buffer. Subsequently, non-specific sites were blocked by the addition of 100 µl blocking buffer to each well. Plates were covered and incubated for one h at 37°C. Subsequently, 10 µl of standard (4 pg – 2048 pg cortisol/10 µl assay buffer or 10 µl of twice-diluted plasma was added to designated wells. Non-specifics and B_0 wells received 10 µl assay buffer. After the addition of standards and samples, 333 Bq of ^{3}H-hydrocortisone (PerkinElmer, USA, 1:10,000 in assay buffer) solution was added to each well. Plates were incubated for four h at room temperature, or stored overnight at 4ºC. The plates were then washed three times with wash buffer. After the final wash step, all wells received 200 µl of 'Optiphase hisafe-3 scintillation liquid' (PerkinElmer, USA) and were covered. Beta-emission was quantified by a 3 min count per well using a Microbeta Plus (Wallac/PerkinElmer, USA). Inter- and intra-assay variations were 12.5 and 3.5%, respectively.

2.5 *In situ* hybridization

In situ hybridization (ISH) for *cfos* and *bdnf* transcripts was performed on parallel sections for 7 fish per treatment. For each fish, the telencephalon was sectioned transversely onto one Superfrost Ultra Plus slide (Menzel-Gläser) using a cryostat (Leica CM 3050) at -24°C. Sections were 14 µm thick and spaced 90 µm apart. Slides were dried at 60°C for 10 min and subsequently stored at -80°C until further analysis. The ISH digoxigenin-labeled probes were made according to Vindas et al. (2017) and were 906 and 485 nucleotides long for *cfos* and *bdnf*, respectively. Forward ACTCCGCTTTCAACACCGAC and reverse TGTAGAGAGGCTCCCAGTCC and forward TCACAGACACGTTTGAGCAGGTGA and reverse ATGCCTCTTGTCTATTCCACGGCA primers were used for *cfos* and *bdnf* probes, respectively. The ISH protocol was conducted according to Ebbesson et al. (2011). Slides were mounted in 70% glycerol in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl.

For both *cfos* and *bdnf*, all 28 slides were stained simultaneously in the same Coplin staining jars in random positions to avoid differences in coloration due to handling effects. Alignment of the probe sequences (Supplementary File 1) in BLAST revealed a 100% similarity with several predicted *Salmo salar bdnf* transcripts (XM014175921.1 and others) and 99% similarity with the predicted *Salmo salar cfos* transcript (XM014206157.1). Both sense and antisense probes were tested to confirm specific labeling of target genes (Supplementary Figure 1).

2.6 Quantification of labeled cells

After ISH, slides were photographed using an Axio Scan.Z1 slide scanner (Zeiss) at 20× magnification. Labeled *cfos* and *bdnf* cells were quantified using the Fiji platform (Schindelin et al., 2012, RRID:SCR_002285) in ImageJ2 (Rueden et al., 2017, RRID:SCR003070). Brain regions were identified using several salmonid stereotaxic atlases (Navas et al., 1995; Carruth et al., 2000; Northcutt, 2006) and transcript-positive cells were counted in the dorsolateral (both the dorsal and ventral subregions; Dld and Dlv, respectively) and dorsomedial (both the dorsal and ventral subregions; Dmd and Dmv, respectively) pallium, as well as in the ventral part of the ventral telencephalon (Vv; see Figure 1 for an overview of the subregions). An Image J macro script was developed to semi-automate quantification of labeled cells (Supplementary File 2). In short, images were converted into grayscale (8 bit), the area of interest was manually selected and the black & white threshold was adjusted within the range of 145 and 190 to match the labeled cells in the original image. Then, all labeled cells that measured between 15-500 pixels were counted using the 'Analyze Particles' command. Example images of the semi-automated quantification method are provided in Supplementary Figure 2. For each section, the total number of transcript-labeled cells was counted in both the entire Dl and Dm, as well as within their respective dorsal and ventral subregions (Dld and Dlv, Dmd and Dmv), to elucidate subregion-specific expression patterns and to allow for comparisons with previous studies (e.g. Vindas et al., 2017). The number of labeled cells was quantified as described by Vindas et al. (2018) and Moltesen et al. (2016). In short, the number of transcript-expressing cells was counted within each subregion for both lobes in each section (in which interest areas were found). Labeled cells were counted in 9.0 ± 1.4 (mean ± SD) telencephalon sections per fish and because the number of brain sections differed per fish, we corrected for the number of counted sections by calculating the average number of labeled cells per section for each subregion. These average numbers of labeled cells per section in each area were used in the statistical analysis. Samples were quantified in random order and the experimenter did not know the identity of the samples at time of quantification.

2.7 Statistical analyses

Two-way analysis of variance (ANOVA) was used to compare fork length, body mass, plasma cortisol levels and ISH cell counts, with origin (wild *vs.* hatchery) and treatment (basal *vs.* stress) as independent variables. The fish telencephalon consists of two lobes (Fig. 1). To test whether lateralization preferences occurred (i.e. different neural responses in the left vs. right telencephalic lobe), the labeled cells were quantified in each lobe separately and for each area of interest, we tested if the left and right cell counts were statistically different from each other (Spearman's ρ). Because we did not find any significant differences in labeled cell numbers between the two lobes, the cell quantifications of the two lobes were pooled together for further statistical analysis and the absolute number of transcript-expressing cells were compared between treatments. Models were assessed by their capacity to explain the variability, and the interaction effects between treatment and conditions were

accepted or rejected according to total model "lack of fit" probabilities. Upon inspection of the diagnostic residual plots, all ISH cell counts and cortisol values were ^{10}log transformed before statistical analysis. Tukey-Kramer honestly significant difference (HSD) post-hoc tests were conducted for brain areas that showed both a significant origin and treatment effect or a significant interaction effect, in order to elucidate differences between groups. Individual data points are shown, as well as the mean ± standard error of the mean (SEM).

3 Results

3.1 Body size and plasma control

As expected, wild fish were significantly smaller (fork length: 81 ± 2 *vs.* 112 ± 1 mm; $p < 0.0001$) and weighed less (6.7 ± 0.6 *vs.* 16.8 ± 0.8 g; $p < 0.0001$) than hatchery-reared fish. Fork length ($p = 0.68$) and body mass ($p = 0.94$) did not significantly differ between stressed and basal fish. Basal plasma cortisol levels were approximately 3.6 ng ml^{-1} for both hatchery and wild parr (Fig. 2). The 30-minute confinement stress significantly elevated plasma cortisol levels to 24.3 ± 4.5 and 20.8 ± 2.6 ng ml^{-1} in hatchery and wild fish, respectively (treatment effect: $p < 0.0001$). No origin or interaction effect was found ($p = 0.76$ and $p = 0.61$, respectively).

3.2 Expression of *cfos* and *bdnf*

3.2.1 The dorsolateral pallium and its subregions

In situ hybridization analysis of *cfos* in the Dl as a whole (Fig. 3A) revealed a significant origin ($p = 0.046$), treatment ($p < 0.0001$) and interaction effect ($p = 0.021$), with a significantly higher absolute number of *cfos*-labeled cells post-stress in both hatchery ($p < 0.0001$) and wild ($p = 0.0027$) fish, compared to basal conditions. In addition, post-stress hatchery fish had a higher number of *cfos*-labeled cells in the Dl compared to post-stress wild fish ($p = 0.017$). In the Dld (Fig. 3B), a treatment effect showed overall more *cfos*-labeled cells in response to stress compared to basal conditions ($p = 0.0071$). No effect of origin was found ($p = 0.091$). The Dlv (Fig. 3C) showed a similar pattern as the whole Dl, with a significant origin ($p = 0.041$), treatment ($p < 0.0001$) and interaction effect ($p = 0.0038$). Post-hoc analysis revealed higher *cfos* expression in response to stress for both hatchery ($p < 0.0001$) and wild ($p < 0.0001$) individuals, compared to values at basal conditions. Furthermore, post-stress hatchery fish had a higher number of *cfos*-positive cells in the Dlv compared to post-stress wild individuals ($p = 0.0045$).

In situ hybridization analysis of *bdnf* in the Dl as a whole (Fig. 3D) and in the Dld (Fig. 3E) revealed no significant origin or treatment effects. Meanwhile, there was a significant origin effect ($p = 0.0074$) in the Dlv (Fig. 3F), with hatchery fish showing overall higher numbers of *bdnf*-labeled cells compared to wild fish.

3.2.2 The dorsomedial pallium and its subregions

There was a significant treatment effect on *cfos* expression in the Dm, Dmd and Dmv ($p < 0.0001$ in all areas; Fig. 4A-C), showing a higher *cfos* transcript abundance in stressed fish. No origin effects were found for the Dm, Dmd or the Dlv.

Expression of *bdnf* showed a significant origin ($p = 0.020$, $p = 0.014$ and $p = 0.049$) and treatment ($p = 0.0025$, $p = 0.0091$ and $p = 0.025$) effect for the Dm (Fig. 4D), Dmd (Fig. 4E) and Dmv (Fig. 4F) respectively, where hatchery fish showed an overall higher *bdnf* abundance compared to wild individuals and post-stress *bdnf* expression was higher

compared to values at basal levels. Tukey-Kramer HSD post hoc tests revealed that in the Dm, the wild group at basal conditions showed significantly lower *bdnf* expression compared to the wild stressed ($p = 0.0129$) and hatchery stressed ($p = 0.0017$) groups, but not to the hatchery basal group ($p = 0.053$). In the Dmd, the wild basal group had a significantly lower number of *bdnf*-labeled cells compared to all three other groups ($p = 0.038$, $p = 0.0032$, $p = 0.027$ for wild basal *vs.* hatchery basal, hatchery stressed and wild stressed, respectively). In the Dmv, the wild basal group showed a significantly lower number of *bdnf*-labeled cells than the hatchery stressed group ($p = 0.0435$) and no other significant differences were found between groups.

3.2.3 The ventral part of the ventral telencephalon

In the Vv, *cfos* (Fig. 5A) expression was significantly elevated overall in response to stress ($p < 0.0001$), while no significant effects of origin were observed ($p = 0.12$).

The number of *bdnf*-labeled cells (Fig. 5B) in the Vv was overall significantly higher in hatchery fish ($p < 0.0001$) compared to wild individuals and no treatment effects were found ($p = 0.22$).

Figure 6 depicts representative examples of ISH images that were used for the quantification analysis.

4 Discussion

We found distinct differences in region-specific expression of *cfos* and *brain-derived neurotrophic factor* (*bdnf*) in the telencephalon of hatchery-reared and wild Atlantic salmon parr under basal and acute stress conditions. While the stressor resulted in increased *cfos* abundance in all fish, hatchery-reared individuals showed a significantly stronger increase in *cfos*-positive cells than wild fish in the ventral part of the dorsolateral pallium (Dlv). Transcript abundance of *bdnf* increased in response to acute stress in the dorsal part of the dorsomedial pallium (Dmd) of wild fish, but not in that of hatchery-reared individuals. Thus, our findings demonstrate that neuronal activity and neural plasticity in Atlantic salmon is dependent on both origin (*i.e*. wild or hatchery-reared) and treatment conditions (*i.e*. basal or post-acute stress) and that these processes differ in a region-specific manner. To our knowledge, we are the first to map neuronal differences between wild and hatchery-reared fish within telencephalic subregions and our results provide novel insights into the neurological foundation that could underlie the differences in behavior, and stocking success, between wild and hatchery-reared fish.

Plasma cortisol levels in stressed parr increased approximately 6-fold compared to controls and the range of average plasma cortisol concentrations found in this study (3-25 ng ml^{-1}) was within the range of those previously reported for non-migratory Atlantic salmon parr (Carey and McCormick, 1998; McCormick et al., 2000; Madaro et al., 2015; Madaro et al., 2016). Because hatchery-reared fish are subject to human disturbance more frequently than wild fish, we had hypothesized that hatchery fish would habituate more easily to stress and therefore show a mitigated cortisol response to a stressor. However, hatchery-reared and wild salmon showed no differences in plasma cortisol concentrations at either basal or post-stress conditions. Similar plasma cortisol levels for wild and hatchery-reared salmonids at 30 minutes post-stress have previously been reported for rainbow trout (*Oncorhynchus mykiss*) (Woodward and Strange, 1987), suggesting that the magnitude of the immediate cortisol response to acute stress is not affected by hatchery rearing. However, several studies report

higher plasma cortisol concentrations in wild salmonids (rainbow trout, Coho salmon (*O. kisutch*) and Chinook salmon (*O. tshawytscha*)) or salmon reared in semi-natural rearing environments (*O. tshawytscha*) compared to hatchery-reared conspecifics at 1-12 hours post-stress, suggesting that recovery of cortisol to baseline levels is slower in wild individuals (Woodward and Strange, 1987; Salonius and Iwama, 1993; Madison et al., 2015). These findings thus suggest that, despite the fact that we observed a similar cortisol response in wild and hatchery-reared fish at 30 minutes post-stress, it is possible that hatchery individuals recover from the acute stress more quickly, which would indicate that the hatchery environment alters the long-term endocrine stress response. Further studies should confirm this hypothesis by assessing cortisol peak levels, as well as recovery duration, in wild *vs.* hatchery Atlantic salmon populations, to determine the effect of hatchery rearing on stress coping.

The immediate early gene *cfos* is a robust marker for recent neural activity (Okuno, 2011). Within specific neuronal populations, the *cfos* gene is relatively little expressed at basal levels but when neurons are stimulated, *cfos* expression is rapidly increased with mRNA levels typically peaking between 15 and 30 minutes post activation (Hoffman et al., 1993; Pavlidis et al., 2015). Acute stress can increase neuronal *cfos* expression in a variety of animals, including rats (Cullinan et al., 1995; Rosen et al., 1998), zebrafish (*Danio rerio*, Pavlidis et al., 2015), gilthead seabream (*Sparus aurata*, Vindas et al., 2018) and Atlantic salmon (Vindas et al., 2017). Our findings corroborate that acute stress increases *cfos* expression in the Dl, Dm and Vv of teleost fish (Vindas et al., 2017; Vindas et al., 2018). Increased *cfos* expression post-acute stress has also been reported in mammalian limbic areas, including in brain regions which are functionally equivalent to the fish Dl, Dm and Vv (Cullinan et al., 1995). Recent studies have suggested that the Dlv, not the Dld, bears most resemblance to the mammalian hippocampus (Broglio et al., 2015). Therefore, we quantified *cfos* expression separately in the dorsal and ventral subregions of the Dl and our observation that *cfos* shows a different expression pattern in the Dlv (treatment, origin and interaction effect) compared to the Dld (treatment effect only) supports the hypothesis that the Dld and Dlv are associated with the regulation of different processes. Research on mice has shown that a fear conditioning stimulus increases CFOS expression in hippocampal cells, and when these same cells are reactivated through optogenetic stimulation the mice display freezing behavior, demonstrating that hippocampal CFOS expression is involved with neural activity associated with fear memory storage and retrieval (Liu et al., 2012). We observed that reared fish showed a greater increase of *cfos* expression in the Dlv in response to acute stress compared to wild individuals. Reared salmonids often show reduced antipredator performance compared to wild conspecifics (Huntingford, 2004). As the hippocampus plays an important role in mammalian fear memory and retrieval, and we observe different responsiveness of the Dlv to confinement stress between hatchery-reared and wild fish, it would be interesting to assess whether wild and reared fish would also display differences in neural activation in the Dlv in a fear-conditioning test, and how this may relate to important behavioural paradigms such as antipredator behaviour. Finally, the Dlv also plays an important role in spatial memory. That is, lesions in the Dlv result in place-memory deficits in goldfish (*Carrasius* auratus, Rodríguez et al., 2002; Broglio et al., 2010) in a similar way that lesions of the hippocampus reduce the navigating capacity of mammals (Hampton et al., 2004). Therefore, it is likely that the Dlv is important for navigating between natural foraging grounds. In this context, it would be interesting to examine whether the difference in post-stress activation of the Dlv that we found between wild and hatchery-reared fish is associated with their ability to navigate, learn and retrieve memories on foraging patches and prey abundance, particularly in a risky environment (*e.g.* under threat from predators).

Brain-derived neurotrophic factor (Bdnf) is a protein from the neurotrophin family that promotes synaptic plasticity, long-term potentiation, neurogenesis and cell survival (Mattson et al., 2004; Pang et al., 2004; Gray et al., 2013). In the whole fish brain, *bdnf* mRNA is generally upregulated in response to acute stress (Pavlidis et al., 2015) and downregulated after chronic stress (Tognoli et al., 2010). Mammalian studies show that changes in *BDNF* expression in response to external stimuli are region-specific within the central nervous system. For example, while both chronic and acute stress result in a significant elevation of BDNF protein abundance in the mammalian amygdala, stress can decrease BDNF levels in the hippocampus (Gray et al., 2013). While mammalian studies almost exclusively study region-specific expression patterns of *BDNF*, studies on teleosts often target *bdnf* expression in the whole brain or macro-brain regions such as the whole telencephalon or whole cerebellum (Tognoli et al., 2010; Johansen et al., 2012; Pavlidis et al., 2015). In these teleostean studies, acute stress increased *bdnf* expression in the whole brain of zebrafish (Pavlidis et al., 2015), while it did not alter *bdnf* transcript abundance in the whole telencephalon of rainbow trout (Johansen et al., 2012) nor in the whole brain of European sea bass, *Dicentrarchus labrax* (Tognoli et al., 2010). Interestingly, while we did not find a change in *bdnf* expression in the Dl and Vv in response to stress in any of our study groups, consistent with the findings of Johansen et al. (2012) and Tognoli et al. (2010), we did find significantly more *bdnf*-labeled cells in the whole of the Dm of wild stressed individuals, supporting the findings by Pavlidis et al. (2015). Furthermore, the increase in post-stress *bdnf* expression in the Dm is in agreement with the finding that BDNF abundance is increased post-stress in the mammalian amygdala (Gray et al., 2013). This result is interesting to study further, particularly because this emotional/stress reactivity center may play a significant role in predator recognition and negative stimuli avoidance conditioning (Portavella et al., 2004). Additionally, this finding further demonstrates that in salmon, as has been shown earlier (Vindas et al., 2017), targeting neuronal subregions can reveal expression patterns that escape detection when studying whole brains or whole macro-brain areas such as the entire telencephalon. Interestingly, as with *cfos* expression in the Dl, the dorsal (Dmd) and ventral (Dmv) neural populations of the Dm in wild fish showed different *bdnf* expression profiles. That is, while acute stress increased *bdnf* expression in the Dmd, it did not in the Dmv. This observation raises the possibility that, similar to what has been proposed for the neural subpopulations of the Dl, the dorsal and ventral subregions of the Dm have different functionalities also, as suggested by preliminary work by Broglio (pers. comm.). Finally, even though hatchery-reared fish did not show any increase in *bdnf* in response to stress, we observed that this group showed an overall higher expression of *bdnf* in the Dm, the Vv and the Dlv, compared to wild individuals, with the highest number of *bdnf*-labeled cells present in the Dm, which plays an important role in learning under fear and stress (Portavella et al., 2004; Vargas et al., 2009; O'Connell and Hofmann, 2011). Under hatchery conditions, fish regularly experience disturbances (*e.g.* tank cleaning, grading, vaccination, transport etc.) at unpredictable intervals. It is conceivable that these stressors periodically trigger *bdnf* transcription in the Dm of hatchery fish, effectively elevating basal *bdnf* expression levels in this subregion. The Dm shares reciprocal neuronal connections with the Vv, which in turn connects to the Dlv (Folgueira et al., 2004a; b; Northcutt, 2006). Notably, mammalian research has demonstrated that the amygdala and hippocampus play an important regulatory role in the hypothalamo-pituitary-adrenal (HPA) axis (McEwen, 2003), and that the lateral septum and amygdala are both part of a circuit involved with stress-induced anxiety behavior (Anthony et al., 2014). Extrapolating our results to these mammalian findings, we propose that the frequent disturbances associated with life in an anthropogenic environment (*i.e.* hatchery rearing) increases *bdnf* expression at basal conditions in the Dm, which in turn promotes *bdnf* expression in the Dl and Vv through neural circuits that are involved in the

stress axis. Together with the fact that hatchery-reared fish did not show an increase in *bdnf* to stress, and the fact that increased BDNF levels are linked to a higher learning performance in mammals (Vaynman et al., 2004), our results may show a potential reduced capacity for learning performance in hatchery-reared fish under acute stressful conditions, which would affect their post-release survival, particularly in risky environments (*e.g.* under predator pressure). Future studies should examine the learning performance and *bdnf* transcription of individuals under stress and non-stress conditions, to elucidate whether our observation that there are different *bdnf* expression patterns between wild and hatchery fish in the Dm affects their emotional learning response.

To our knowledge, no other studies had ever compared neurobiological markers between wild and hatchery-reared salmonids using a detailed region-specific approach. For this reason, we selected *in situ* hybridization as our methodology, since it allows for the visualization of transcript abundance of target genes in the entire brain, providing a clear overview of which neural subpopulations can be of interest. The disadvantage of using ISH to quantify expression of neurobiological markers is that the quantification process in our analysis is binary: cells are either classified as expressing or non-expressing, while the relative transcript abundance within the cells is not considered. In order to map gene expression patterns in a more quantitative manner, future studies should perform microdissections of the relevant forebrain subregions and subsequently quantify the transcript abundance by quantitative polymerase chain reaction (qPCR), as we have previously done when studying the neurobiological component of coping styles in Atlantic salmon (Vindas et al., 2017). By using ISH in the current study, we were able to compare region-specific expression of neurobiological markers between wild and hatchery-reared fish on the most detailed scale to date. The studied neuronal subpopulations are involved in learning processes and stress reactivity and thus provide an important insight in how neural plasticity may drive behavioral differences between wild and hatchery-reared fish.

In conclusion, we demonstrate that the rearing environment is an important driver of neuronal wiring in the telencephalon of Atlantic salmon parr. We show novel data on expression of neuroplasticity markers within specific neuronal subregions in wild and hatchery-reared fish and this approach has unveiled stress-related expression patterns that have previously escaped detection (i.e. when studying larger brain areas; Johansen et al., 2012; Pavlidis et al., 2015). The specific brain areas mapped in the current study are associated with cognitive processing capacity (specifically stress reactivity, associative learning and emotional learning) and may therefore play an important role in the behavioral differences that are observed between wild and hatchery-reared teleosts. A better understanding of how the rearing environment affects the neurological and behavioral plasticity of captive animals will help with the future design of innovative hatchery technologies that produces well-adapted salmonids that can thrive after stocking. In addition, these results provide further insight into mechanisms of the central nervous system associated with behavioral processing and coping in vertebrates and provides focal areas which should be studied further to elucidate how animals react to, and interact with, their environment.

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6 Author Contributions

DM, KK, IM and MV contributed conception and design of the study; DM and KK collected the samples; DM, MG and MV performed laboratory analyses; DM and MV conducted statistical analysis; DM and MV wrote the first draft of the manuscript. All authors contributed significantly to manuscript revision and approved the final version.

7 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

8 Data Availability Statement

All relevant data are within the paper and its supplementary material.

9 Supplementary Material

Supplementary Figure 1

Supplementary Figure 2

Supplementary File 1

Supplementary File 2

10 References

Anthony, T.E., Dee, N., Bernard, A., Lerchner, W., Heintz, N., and Anderson, D.J. (2014). Control of stress-induced persistent anxiety by an extra-amygdala septohypothalamic circuit. *Cell* 156(3)**,** 522-536. doi: 10.1016/j.cell.2013.12.040.

Araki, H., and Schmid, C. (2010). Is hatchery stocking a help or harm? *Aquaculture* 308**,** S2-S11. doi: 10.1016/j.aquaculture.2010.05.036.

Aubin-Horth, N., Letcher, B.H., and Hofmann, H.A. (2005). Interaction of rearing environment and reproductive tactic on gene expression profiles in Atlantic salmon. *J. Hered.* 96(3)**,** 261-278. doi: 10.1093/jhered/esi030.

Broglio, C., Martín-Monzón, I., Ocaña, F., Gómez, A., Durán, E., Salas, C., et al. (2015). Hippocampal pallium and map-like memories through vertebrate evolution. *J. Behav. Brain. Sci* 5(3)**,** 109. doi: 10.4236/jbbs.2015.53011.

Broglio, C., Rodríguez, F., Gómez, A., Arias, J.L., and Salas, C. (2010). Selective involvement of the goldfish lateral pallium in spatial memory. *Behav. Brain Res.* 210(2)**,** 191-201. doi: 10.1016/j.bbr.2010.02.031.

Brown, G.E., Ferrari, M.C.O., and Chivers, D.P. (2013). Adaptive forgetting: why predator recognition training might not enhance poststocking survival. *Fisheries* 38(1)**,** 16-25. doi: 10.1080/03632415.2013.750133.

Bshary, R., and Brown, C. (2014). Fish cognition. *Curr. Biol.* 24(19)**,** R947-R950. doi: 10.1016/j.cub.2014.08.043.

543 Carey, J.B., and McCormick, S.D. (1998). Atlantic salmon smolts are more responsive to an
544 acute handling and confinement stress than parr. *Aquaculture* 168(1)**,** 237-253. doi:
545 10.1016/S0044-8486(98)00352-4.
546 Carruth, L.L., Jones, R.E., and Norris, D.O. (2000). Cell density and intracellular
547 translocation of glucocorticoid receptor-immunoreactive neurons in the kokanee salmon
548 (*Oncorhynchus nerka kennerlyi*) brain, with an emphasis on the olfactory system. *Gen.*
549 *Comp. Endocrinol.* 117(1)**,** 66-76. doi: 10.1006/gcen.1999.7391.
550 Cullinan, W.E., Herman, J.P., Battaglia, D.F., Akil, H., and Watson, S.J. (1995). Pattern and
551 time course of immediate early gene expression in rat brain following acute stress.
552 *Neuroscience* 64(2)**,** 477-505. doi: 10.1016/0306-4522(94)00355-9.
553 Dunlap, K.D., Silva, A.C., and Chung, M. (2011). Environmental complexity, seasonality and
554 brain cell proliferation in a weakly electric fish, *Brachyhypopomus gauderio*. *J. Exp. Biol.*
555 214(5)**,** 794-805. doi: 10.1242/jeb.051037.
556 Ebbesson, L.O., and Braithwaite, V.A. (2012). Environmental effects on fish neural plasticity
557 and cognition. *J. Fish Biol.* 81(7)**,** 2151-2174. doi: 10.1111/j.1095-8649.2012.03486.x.
558 Ebbesson, L.O.E., Nilsen, T.O., Helvik, J.V., Tronci, V., and Stefansson, S.O. (2011).
559 Corticotropin-releasing factor neurogenesis during midlife development in salmon:
560 genetic, environmental and thyroid hormone regulation. *J. Neuroendocrinol.* 23(8)**,** 733-
561 741. doi: 10.1111/j.1365-2826.2011.02164.x.
562 Folgueira, M., Anadón, R., and Yáñez, J. (2004a). An experimental study of the connections
563 of the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). I: Olfactory bulb and
564 ventral area. *J. Comp. Neurol.* 480(2)**,** 180-203. doi: 10.1002/cne.20340.
565 Folgueira, M., Anadón, R., and Yáñez, J. (2004b). Experimental study of the connections of
566 the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). II: Dorsal area and preoptic
567 region. *J. Comp. Neurol.* 480(2)**,** 204-233. doi: 10.1002/cne.20341.
568 Gorissen, M., Bernier, N.J., Manuel, R., de Gelder, S., Metz, J.R., Huising, M.O., et al.
569 (2012). Recombinant human leptin attenuates stress axis activity in common carp
570 (*Cyprinus carpio L.*). *Gen. Comp. Endocrinol.* 178(1)**,** 75-81. doi:
571 10.1016/j.ygcen.2012.04.004.
572 Gray, J.D., Milner, T.A., and McEwen, B.S. (2013). Dynamic plasticity: the role of
573 glucocorticoids, brain-derived neurotrophic factor and other trophic factors *Neuroscience*
574 239**,** 214-227. doi: 10.1016/j.neuroscience.2012.08.034.
575 Hampton, R.R., Hampstead, B.M., and Murray, E.A. (2004). Selective hippocampal damage
576 in rhesus monkeys impairs spatial memory in an open-field test. *Hippocampus* 14(7)**,** 808-
577 818. doi: 10.1002/hipo.10217.
578 Hoffman, G.E., Smith, M.S., and Verbalis, J.G. (1993). c-Fos and related immediate early
579 gene products as markers of activity in neuroendocrine systems. *Front. Neuroendocrinol.*
580 14(3)**,** 173-213. doi: 10.1006/frne.1993.1006.
581 Huntingford, F.A. (2004). Implication of domestication and rearing conditions for the
582 behaviour of cultivated fishes. *J. Fish Biol.* 65**,** 122-142. doi: 10.1111/j.1095-
583 8649.2004.00562.x.
584 Johansen, I.B., Sorensen, C., Sandvik, G.K., Nilsson, G.E., Hoglund, E., Bakken, M., et al.
585 (2012). Neural plasticity is affected by stress and heritable variation in stress coping style.
586 *Comp. Biochem. Physiol. Part D* 7(2)**,** 161-171. doi: 10.1016/j.cbd.2012.01.002.
587 Johnson, J.H., McKeon, J.E., and Dropkin, D.S. (1996). Comparative diets of hatchery and
588 wild Atlantic salmon smolts in the Merrimack river. *N. Am. J. Fish. Manage.* 16(2)**,** 440-
589 444. doi: 10.1577/1548-8675(1996)016<0440:cdohaw>2.3.co;2.
590 Johnsson, J.I., Brockmark, S., and Näslund, J. (2014). Environmental effects on behavioural
591 development consequences for fitness of captive-reared fishes in the wild. *J. Fish Biol.*
592 85(6)**,** 1946-1971. doi: 10.1111/jfb.12547.

Jonsson, B., and Jonsson, N. (2009). "Restoration and enhancement of salmonid populations and habitats with special reference to Atlantic salmon", in: *Challenges for Diadromous Fishes in a Dynamic Global Environment, American Fisheries Society Symposium*, eds. A.J. Haro, T.S. Avery & K.L. Beal), 497-535.

Kalueff, A.V., Stewart, A.M., Kyzar, E.J., Cachat, J., Gebhardt, M., Landsman, S., et al. (2012). Time to recognize zebrafish 'affective' behavior. *Behaviour* 149(10-12)**,** 1019-1036. doi: 10.1163/1568539X-00003030.

Liu, X., Ramirez, S., Pang, P.T., Puryear, C.B., Govindarajan, A., Deisseroth, K., et al. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* 484**,** 381-385. doi: 10.1038/nature11028.

Long, C.W., McComas, J.R., and Monk, B.H. (1977). Use of salt (NaCl) water to reduce mortality of Chinook salmon smolts, *Oncorhynchus Tshawytscha*, during handling and hauling. *Mar. Fish. Rev.* 39(7)**,** 6-9.

Madaro, A., Olsen, R.E., Kristiansen, T.S., Ebbesson, L.O.E., Flik, G., and Gorissen, M. (2016). A comparative study of the response to repeated chasing stress in Atlantic salmon (*Salmo salar* L.) parr and post-smolts. *Comp. Biochem. Physiol., A: Mol. Integr. Physiol.* 192**,** 7-16. doi: 10.1016/j.cbpa.2015.11.005.

Madaro, A., Olsen, R.E., Kristiansen, T.S., Ebbesson, L.O.E., Nilsen, T.O., Flik, G., et al. (2015). Stress in Atlantic salmon: response to unpredictable chronic stress. *J. Exp. Biol.* 218(16)**,** 2538. doi: 10.1242/jeb.120535.

Madison, B.N., Heath, J.W., Heath, D.D., and Bernier, N.J. (2015). Effects of early rearing environment and breeding strategy on social interactions and the hormonal response to stressors in juvenile Chinook salmon. *Can. J. Fish. Aquat. Sci.* 72(5)**,** 673-683. doi: 10.1139/cjfas-2014-0409.

Mattson, M.P., Maudsley, S., and Martin, B. (2004). BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* 27(10)**,** 589-594. doi: 10.1016/j.tins.2004.08.001.

Maynard, D.J., and Trial, J.G. (2013). The use of hatchery technology for the conservation of Pacific and Atlantic salmon. *Rev. Fish Biol. Fish.* 24(3)**,** 803-817. doi: 10.1007/s11160-013-9341-7.

McCormick, S.D., Moriyama, S., and Björnsson, B.T. (2000). Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278(5)**,** R1352-R1361. doi: 10.1152/ajpregu.2000.278.5.R1352.

McEwen, B.S. (2003). Early life influences on life-long patterns of behavior and health. *Ment. Retard. Dev. Disabil. Res. Rev.* 9(3)**,** 149-154. doi: 10.1002/mrdd.10074.

Moltesen, M., Vindas, M.A., Winberg, S., Ebbesson, L., de Lourdes Ruiz-Gomez, M., Skov, P.V., et al. (2016). Cognitive appraisal of aversive stimulus differs between individuals with contrasting stress coping styles; evidences from selected rainbow trout (*Oncorhynchus mykiss*) strains. *Behaviour* 153(13-14)**,** 1567-1587. doi: 10.1163/1568539X-00003405.

Munakata, A., Björnsson, B.T., Jönsson, E., Amano, M., Ikuta, K., Kitamura, S., et al. (2000). Post-release adaptation processes of hatchery-reared honmasu salmon parr. *J. Fish Biol.* 56**,** 163-172. doi: 10.1111/j.1095-8649.2000.tb02092.x.

Navas, J.M., Anglade, I., Bailhache, T., Pakdel, F., Breton, B., Jégo, P., et al. (1995). Do gonadotrophin-releasing hormone neurons express estrogen receptors in the rainbow trout? A double immunohistochemical study. *J. Comp. Neurol.* 363(3)**,** 461-474. doi: 10.1002/cne.903630309.

Northcutt, R.G. (2006). Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *J. Comp. Neurol.* 494(6)**,** 903-943. doi: 10.1002/cne.20853.

O'Connell, L.A., and Hofmann, H.A. (2011). The vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *J. Comp. Neurol.* 519(18)**,** 3599-3639. doi: 10.1002/cne.22735.

Okuno, H. (2011). Regulation and function of immediate-early genes in the brain: Beyond neuronal activity markers. *Neurosci. Res.* 69(3)**,** 175-186. doi: 10.1016/j.neures.2010.12.007.

Olla, B.L., Davis, M.W., and Ryer, C.H. (1998). Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bull. Mar. Sci.* 62(2)**,** 531-550.

Palmé, A., Wennerström, L., Guban, P., Ryman, N., and Laikre, L. (2012). Compromising Baltic salmon genetic diversity - conservation genetic risks assiciated with compensatory releases of salmon in the Balctic Sea. *Havs- och vattenmyndighetens rapport 2012:18***,** 115 pp.

Pang, P.T., Teng, H.K., Zaitsev, E., Woo, N.T., Sakata, K., Zhen, S., et al. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* 306(5695)**,** 487. doi: 10.1126/science.1100135.

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**,** 281-287. doi: 10.1139/cjfas-55-S1-281.

Pavlidis, M., Theodoridi, A., and Tsalafouta, A. (2015). Neuroendocrine regulation of the stress response in adult zebrafish, *Danio rerio*. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 60**,** 121-131. doi: 10.1016/j.pnpbp.2015.02.014.

Portavella, M., Torres, B., and Salas, C. (2004). Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. *J. Neurosci.* 24(9)**,** 2335-2342. doi: 10.1523/jneurosci.4930-03.2004.

Pottinger, T.G., Pickering, A.D., and Hurley, M.A. (1992). Consistency in the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 103(3)**,** 275-289. doi: 10.1016/0044-8486(92)90172-H.

Rodríguez, F., López, J.C., Vargas, J.P., Gómez, Y., Broglio, C., and Salas, C. (2002). Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. *J. Neurosci.* 22(7)**,** 2894-2903.

Rosen, J.B., Fanselow, M.S., Young, S.L., Sitcoske, M., and Maren, S. (1998). Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. *Brain Res.* 796(1)**,** 132-142. doi: 10.1016/S0006-8993(98)00294-7.

Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T., et al. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 18(1)**,** 529. doi: 10.1186/s12859-017-1934-z.

Salonius, K., and Iwama, G.K. (1993). Effects of early rearing environment on stress response, immune function, and disease resistance in juvenile coho (*Oncorhynchus kisutch*) and Chinook salmon (*O. tshawytscha*). *Can. J. Fish. Aquat. Sci.* 50(4)**,** 759-766. doi: 10.1139/f93-087.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9**,** 676. doi: 10.1038/nmeth.2019.

Shors, T.J., Anderson, M.L., Curlik, D.M., and Nokia, M.S. (2012). Use it or lose it: How neurogenesis keeps the brain fit for learning. *Behav. Brain Res.* 227(2)**,** 450-458. doi: 10.1016/j.bbr.2011.04.023.

Singewald, G.M., Rjabokon, A., Singewald, N., and Ebner, K. (2011). The modulatory role of the lateral septum on neuroendocrine and behavioral stress responses. *Neuropsychopharmacology* 36(4)**,** 793-804. doi: 10.1038/npp.2010.213.

693 Stewart, A.M., and Kalueff, A.V. (2012). The developing utility of zebrafish models for
694 cognitive enhancers research. *Curr. Neuropharmacol.* 10(3)**,** 263-271. doi:
695 10.2174/157015912803217323.
696 Thorstad, E.B., and Forseth, T. (2015). Status for norske laksebestander i 2015. *Report of*
697 *Vitenskapelig råd for lakseforvaltning* (Nr. 8)**,** 300 pp.
698 Thorstad, E.B., Uglem, I., Arechavala-Lopez, P., Økland, F., and Finstad, B. (2011). Low
699 survival of hatchery-released Atlantic salmon smolts during initial river and fjord
700 migration. *Boreal Environ. Res.* 16**,** 115-120.
701 Tognoli, C., Rossi, F., Di Cola, F., Baj, G., Tongiorgi, E., Terova, G., et al. (2010). Acute
702 stress alters transcript expression pattern and reduces processing of proBDNF to mature
703 BDNF in *Dicentrarchus labrax*. *BMC Neurosci.* 11(1)**,** 4. doi: 10.1186/1471-2202-11-4.
704 Vargas, J.P., López, J.C., and Portavella, M. (2009). What are the functions of fish brain
705 pallium? *Brain Res. Bull.* 79(6)**,** 436-440. doi: 10.1016/j.brainresbull.2009.05.008.
706 Vaynman, S., Ying, Z., and Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the
707 efficacy of exercise on synaptic plasticity and cognition. *Eur. J. Neurosci.* 20(10)**,** 2580-
708 2590. doi: 10.1111/j.1460-9568.2004.03720.x.
709 Vindas, M.A., Fokos, S., Pavlidis, M., Höglund, E., Dionysopoulou, S., Ebbesson, L.O.E., et
710 al. (2018). Early life stress induces long-term changes in limbic areas of a teleost fish: the
711 role of catecholamine systems in stress coping. *Sci. Rep.* 8(1)**,** 5638. doi: 10.1038/s41598-
712 018-23950-x.
713 Vindas, M.A., Gorissen, M., Höglund, E., Flik, G., Tronci, V., Damsgård, B., et al. (2017).
714 How do individuals cope with stress? Behavioural, physiological and neuronal differences
715 between proactive and reactive coping styles in fish. *J. Exp. Biol.***,** 1524-1532. doi:
716 10.1242/jeb.153213.
717 Woodward, C.C., and Strange, R.J. (1987). Physiological stress responses in wild and
718 hatchery-reared rainbow trout. *Trans. Am. Fish. Soc.* 116(4)**,** 574-579. doi: 10.1577/1548-
719 8659(1987)116<574:PSRIWA>2.0.CO;2.
720

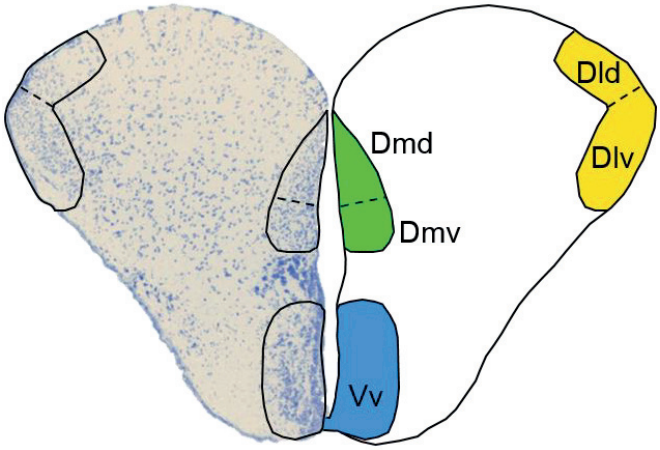
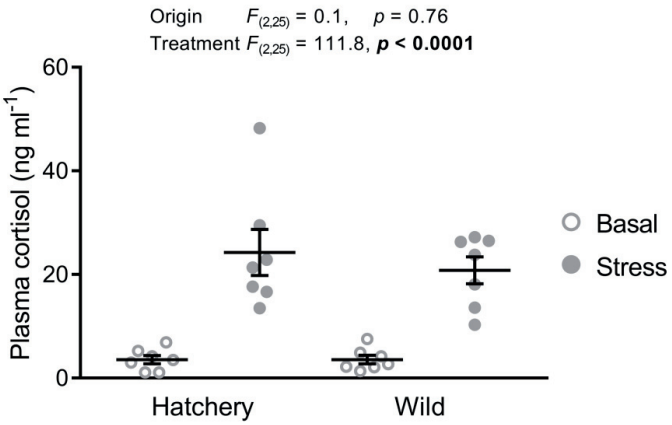


Figure 1. Selected subregions of the telencephalon. A transverse view of the Atlantic salmon telencephalon with a Toluidine Blue-stained left lobe and a schematic representation of the right lobe depicting the location of the dorsal (Dld) and ventral (Dlv) dorsolateral pallium, the dorsal (Dmd) and ventral (Dmv) dorsomedial pallium and the ventral part of the ventral telencephalon (Vv).



728

729 **Figure 2. Acute stress elevates plasma cortisol levels.** Effect of origin (hatchery *vs.* wild)
730 and treatment (basal *vs.* stress) on mean ± SEM plasma cortisol levels of Atlantic salmon
731 parr. Two-way analysis of variance (ANOVA) statistics are displayed in the figure, n = 7 per
732 treatment.

733

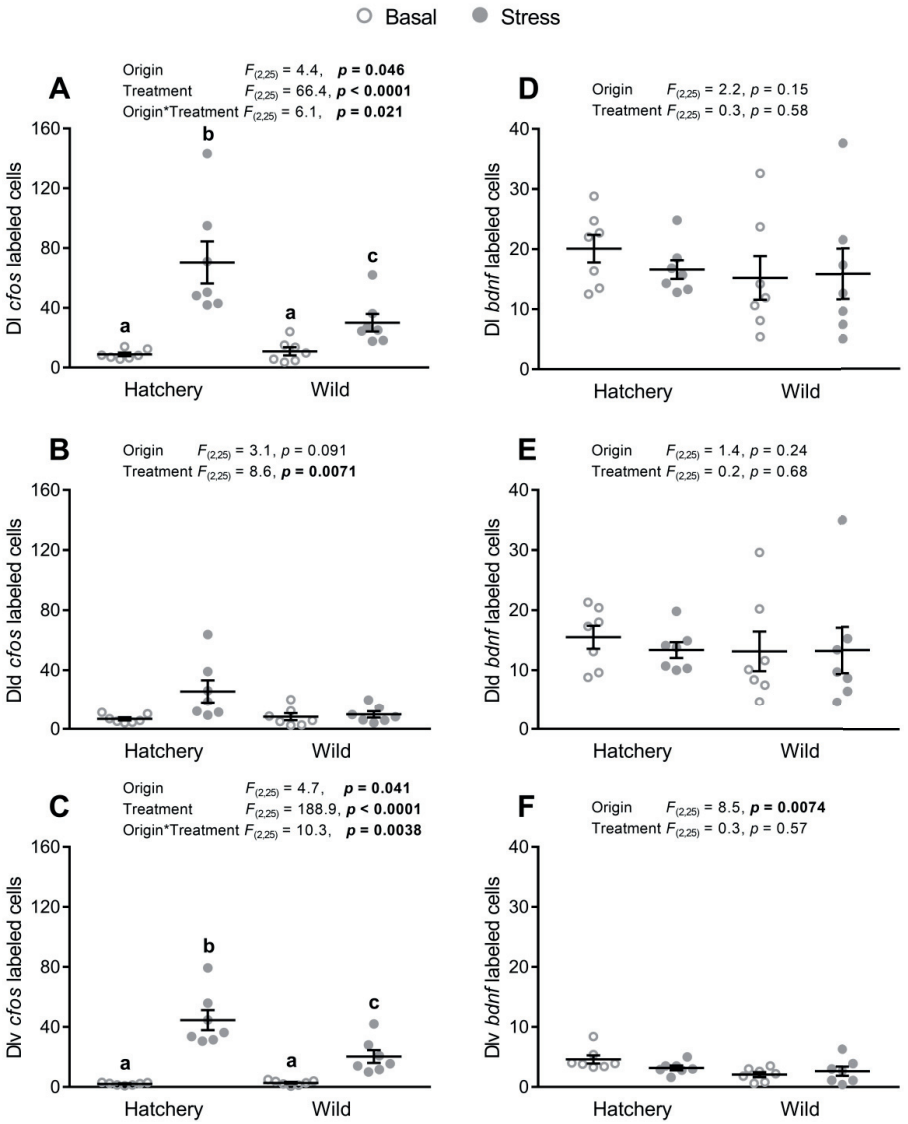


Figure 3. *In situ* hybridization labeled *cfos* and *bdnf* cells in the Dl, Dld and Dlv. Effect of origin (hatchery *vs.* wild) and treatment (basal *vs.* stress) on mean ± SEM expression of *cfos* (**A-C**) and brain-derived neurotrophic factor (*bdnf*; **D-F**) in the entire (dorsal + ventral) dorsolateral pallium (Dl; **A,D**), as well as the dorsal only (Dld; **B,E**) and the ventral only (Dlv; **C,F**) subregions. Two-way analysis of variance (ANOVA) statistics are displayed in each panel, n = 7 per treatment. Groups that do not share a similar lowercase letter are significantly different from one another (Tukey-Kramer HSD post hoc test).

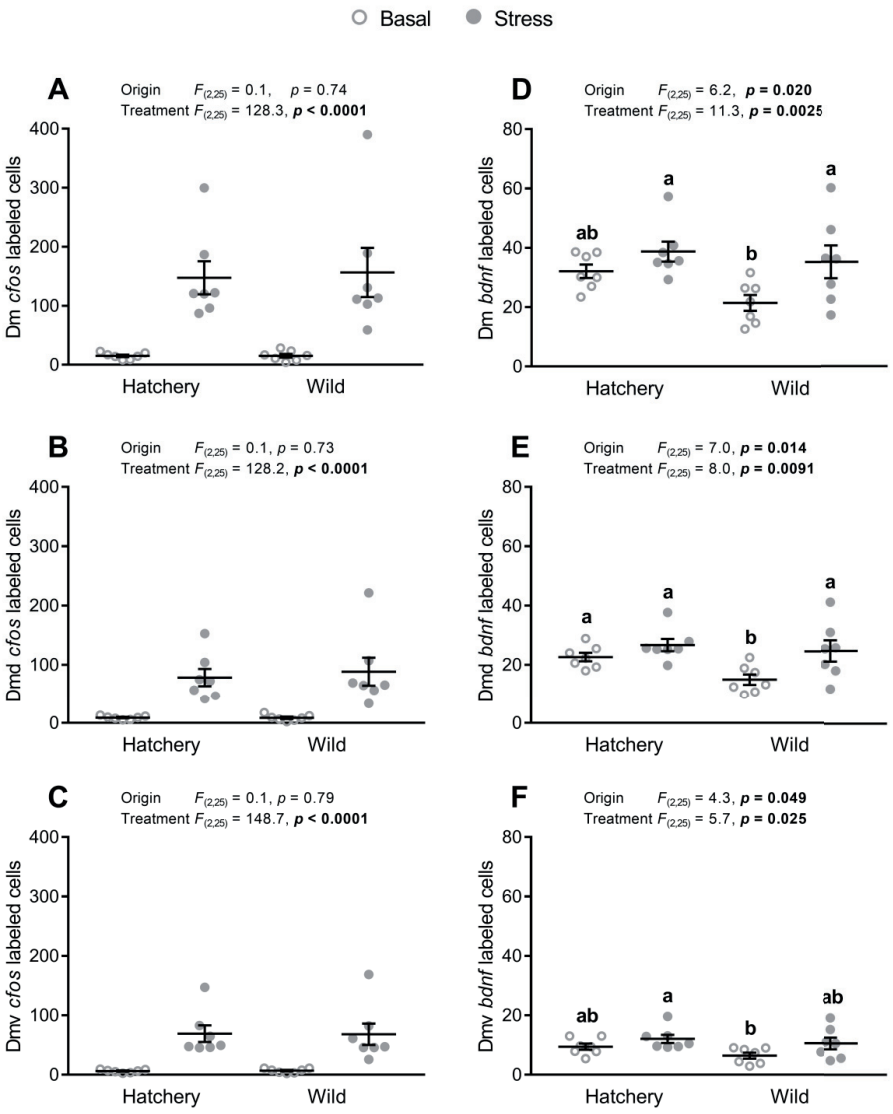


Figure 4. *In situ* hybridization labeled *cfos* and *bdnf* cells in the Dm, Dmd and Dmv. Effect of origin (hatchery *vs.* wild) and treatment (basal *vs.* stress) on mean ± SEM expression of *cfos* (**A-C**) and brain-derived neurotrophic factor (*bdnf*; **D-F**) in the entire (dorsal + ventral) dorsomedial pallium (Dm; A,D), as well as the dorsal only (Dmd; **B,E**) and the ventral only (Dmv; **C,F**) subregions. Two-way analysis of variance (ANOVA) statistics are displayed in each panel, n = 7 per treatment. Groups that do not share a similar lowercase letter are significantly different from one another (Tukey-Kramer HSD post hoc test).

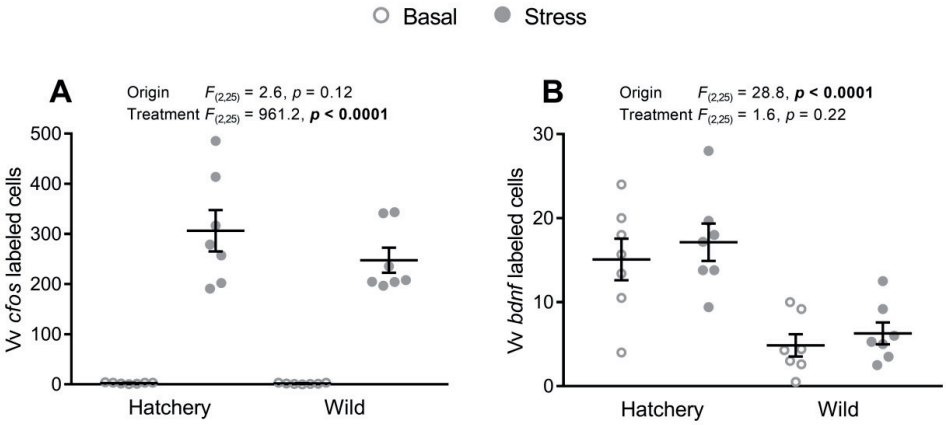


Figure 5. *In situ* hybridization labeled *cfos* and *bdnf* cells in the Vv. Effect of origin (hatchery *vs.* wild) and treatment (basal *vs.* stress) on mean ± SEM expression of *cfos* (**A**) and brain-derived neurotrophic factor (*bdnf*; **B**) in the ventral part of the ventral telencephalon (Vv). Two-way analysis of variance (ANOVA) statistics are displayed in each panel, n = 7 per treatment.

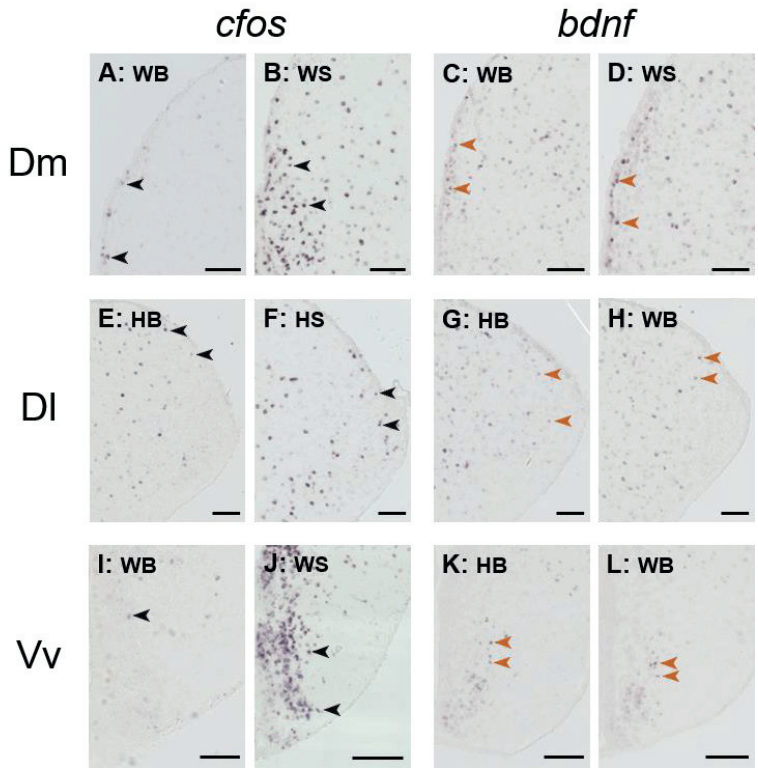


Figure 6. Representative example of *in situ* hybridization of *cfos* and *bdnf;* images used for the quantification analysis. Representative pictures of the expression of *cfos* (**A,B,E,F,I,J**) and brain-derived neurotrophic factor (*bdnf*; **C,D,G,H,K,L**) transcripts (purple cells) in the dorsomedial pallium (Dm; **A-D**), dorsolateral pallium (Dl; **E-H**) and ventral part of the ventral telencephalon (Vv; **I-L**) of wild and hatchery-reared Atlantic salmon parr under basal or after acute stress conditions. WB: wild basal; WS: wild stress; HB: hatchery-reared basal; HS: hatchery-reared stress. Arrows indicate transcript-labeled cells and all scale bars measure 100 μm.

Neurobiology of wild and hatchery-reared Atlantic salmon: how nurture drives neuroplasticity

Daan Mes, Kristine von Krogh, Marnix Gorissen, Ian Mayer, Marco A. Vindas

Supplementary Material

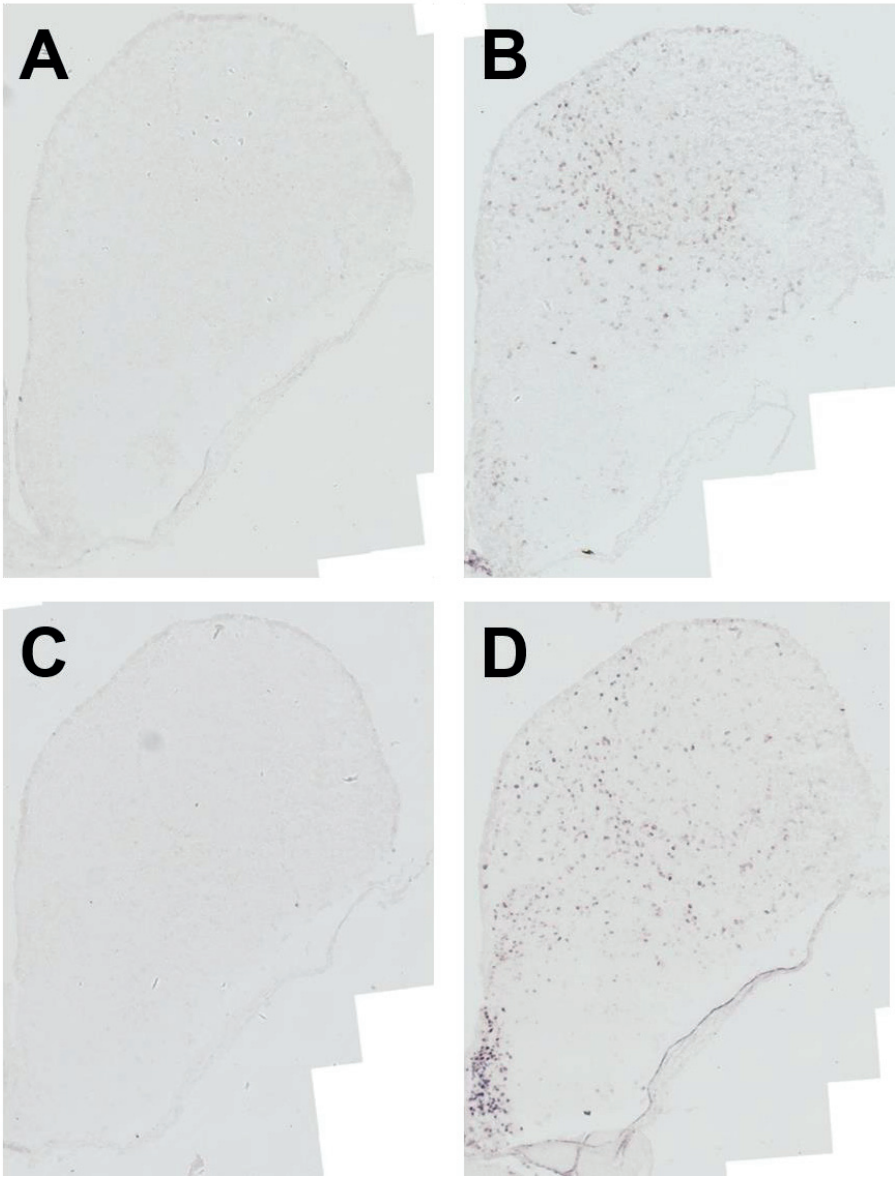


Figure S1. Specificity of the riboprobes was verified by performing the *in situ* hybridization protocol using both sense and antisense probes on a subset of brain samples. Depicted are the sense (**A**) and antisense (**B**) probes for *bdnf* and the sense (**C**) and antisense (**D**) probes for *cfos*.

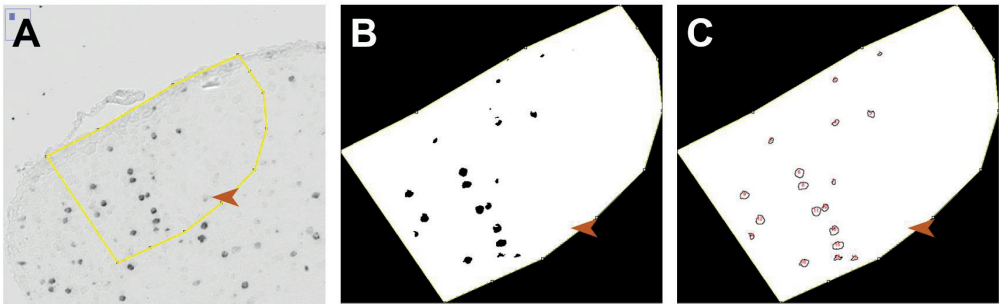


Figure S2. Illustration of several steps of the semi-automated quantification method of transcript-positive cells. The area of interest was selected using the selection tool (**A**), the black & white threshold was set to match the original image as closely as possible (**B**), and all cells that measures between 15-500 pixels were counted by the ImageJ software (**C**). Orange arrows indicate the location of a cell which is labeled too weakly to be quantified by the software after adjusting the black & white threshold in panel **B**.

Supplementary File 1. Sequences of the riboprobes of *bdnf* and *cfos* used for *in situ* hybridization.

***bdnf* (485 bp)**

tgcntGCTCgngCGGCCGCcaGTGTGATGGaTATCTGCAGAATTCGGCTTATGCCTCTT
GTCTATTCCACGGCAGCCCTCCTTTGTGTACCCCATAGGGTTACATTTGGTCTCAT
AAAAGTATTGCTTCAGTTGGCCATTGGGGACAGGGACCTTTTCCAGGACGGTAAC
GGTCTGCCCAGACATGTCTATTGCTGTCTTTTTGTCCACAGCTGTCACCCACTGGC
TAATACTATCACACACACTCAGCTCTCCACGCCGCGACGGGTCAGAATGCCGCCG
CACCCTCATGGACATGTTAGCGGCGTCCAGGTAGTTTTTGTATTCCTCCAGGAGA
AAAAGCAGCGGTGGCTCTAAAGGCACTTGGTTGCTGATCATCACCCGCGATGCAT
ACAGGTCGACATCCTTGGTCTCCGTGGTGACCACAGAGGAAGGACCCCCTCCTCC
CTGGCCCTTGTCAGCCCCAGGCCCCAGCTGAGATGCTTCTCCTTCCACCTCCAAG
AGCTCCTCAATCACCTGCTCAAACGTGTCTGTGAAAGCCGAATTCCAGCACACTG
GCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGGCGTAATCATGGTCA
TAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAG
CCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACAT
TAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCT
GCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGgCGCTCT
TCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGG
TATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGaTAACGC
AGGAAnGAACATGTGAGCAAAnGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG
CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAgCATCACAAAAaT
CGACGCTCAAGTCagaGGTGGCGAAaCCcGaCAGGACTATAAagaTACCAGGCGTTtC
CCccTGGAancTCCcTcGTGcgcTCTCCTGTTCcnaCCcTGCcgcTTACcGGatnCCTGTCcgC
ct

Primers used for cloning:

bdnf F1 ATGCCTCTTGTCTATTCCACGGCA

bdnf R1 TCACAGACACGTTTGAGCAGGTGA
Compl: TCACCTGCTCAAACGTGTCTGTGA

***cfos* (906 bp)**

NNNNNNNNNTACCCTNNCTAAAGGGACTAGTCCTGCAGGTTTAAACGAATTCGC
CCTTTGTAGAGAGGCTCCCAGTCCTGAGTGTACAGGGAGCTGGCCAGATCAACCT
CGGGCACCGACCGGGCCGTCTCCATCTCAGCCTTGGCGAGGAGAGACAGGGACT
CCAGGCAGGCTGTGTCCAGGTCGGCCATCTTGACGTCGGAGACGGAGATGGAGG
CAGTGGACAGGAAGGGACTGCTGGAGAATATGGAGGCAGAGGCTGAGAGGGTG
GAGGTGGACTGGATGGAAGTAAGGGGAGCGGCAGAGCAGGATACCATGCGCTG
AGGCGGGGACAGCTGGATGGAGACCCCGTGGGAGGGCGAGATGGAAGGGAAAG
TCGTGTCCATATCAGAGCGGATCTTGCAGATGGGCTGGTGGGCTGCCAAGATAA
ACTCTAACTTCTCCTTCTCTTTAAGCAGGTTGGCGATGTCGTTCTGGAGAACAGA
CTTCTCTTCCTCCAGCTCGTCGGTTTCACCCTGCAGAGTGTCGGTGAGTTCCTTTC
GCCTGTTGCGGCATTTAGCTGCTGCCTGCTTGTTCCTCTCTCTACGGACTCGCTTC
TTCTCCTCCTCTTCAGGCGAAAGCTGTTCCATTTTGCCTCTGCGCCCAGAGCTGTG
GCCCTTGTTCCTCATGGCTCTAGTGTAGGTTGGGGGGCTGTCGCTGTAGGGATGG
GCTCTGTGAGAAGGTGCCACAGAGGATAGCGGCTGGACCAACCACTGCAGGTCT
GGGCTGGCAGAGATGGCTGTAACAGTAGGGATGAAGGACGGACCACTGGACACT
GAATTCGGGTCTGTGAAGTCCTGAGATTGGGGTGAACCCATGCTGGAGTAAGAT
CCCTCNNGAGAGTTGAAGTAAACCAGCTTGTCGCNNNATGGAGAAGCTGTACTA
CAGCGAGAAGAANANTCACAGTCGGTGTTGAAAGCGGAGTANNNNGAATTCGC
GGCCGCTAAATTCAATTCGCCCTATANTGAGTCGTATTACNATTCACTGGNNGTC
NTTTTACAACGTCNNGACTGGGAAAANNN

Primers used for cloning:

cFosF1: ACTCCGCTTTCAACACCGAC
Compl: GTCGGTGTTGAAAGCGGAGT

cFosR1: TGTAGAGAGGCTCCCAGTCC

Supplementary File 2. The macro script used for quantification of labeled cells using the Fiji platform in ImageJ2.

```
run("Rotate... ");

run("8-bit");

setTool("polygon");

waitForUser

run("Crop");

setBackgroundColor(0, 0, 0);

run("Clear Outside");

run("Threshold...")

waitForUser

run("Convert to Mask");

run("Fill Holes");

run("Watershed");

run("Analyze Particles...", "size=15-1000 pixel show=Outlines clear summarize");
```

II

Effects of environmental enrichment on forebrain neural plasticity and survival success of stocked Atlantic salmon

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[d] Norwegian Institute for Nature Research, Trondheim, Norway

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Fish reared for stocking programs are severely stimulus-deprived compared to their wild conspecifics raised under natural conditions. This leads to reduced behavioural plasticity and low post-release survival of stocked fish. Environmental enrichment can have positive effects on important life-skills, such as predator avoidance and foraging behaviour, but the neural mechanisms underpinning these behavioural changes are still largely unknown. In this study, juvenile Atlantic salmon (*Salmo salar*) were reared in an enriched hatchery environment for seven weeks, after which neurobiological characteristics and post-release survival were compared to fish reared under normal hatchery conditions. Using *in situ* hybridisation and qPCR, we quantified the expression of four neuroplasticity and neurogenesis markers in telencephalic subregions associated with relational memory, emotional learning, and stress reactivity. Aside from lower expression of *brain-derived neurotrophic factor* (*bdnf*) in the Dlv (a region associated with relational memory) of enriched salmon, we observed no significant effects of enrichment on neural plasticity. Exposure to an enriched environment increased post-release survival during a five-month residence in a natural river by 51%. Thus, we demonstrate that environmental enrichment can improve stocking success of Atlantic salmon parr, but this does not appear to be associated with increased neural plasticity in the studied telencephalic subregions.

Introduction

Hatchery and aquaculture rearing environments have a profound impact on fish development and behavioural responses. Compared to fish in the wild, hatchery-reared fish are kept at unnaturally high

densities in a uniform environment and are severely stimulus-deprived in terms of feeding variability, predator exposure and fluctuations in abiotic factors[1]. As a result, current commercial hatchery procedures result in the production of fish that deviate from their wild conspecifics in behavioural[1,2] and neural[3] characteristics. This has implications for production and welfare aspects of fish rearing, in particular within the context of compensatory stocking programs. Millions of hatchery-reared salmonids, primarily Atlantic salmon (*Salmo salar* L.), are released into natural waters in Northern Europe yearly[4,5]. Unfortunately, salmonid stocking is currently characterised by high post-release mortality rates and reared salmon show lower survival compared to wild conspecifics[6-8]. From both an ethical and financial perspective, it is imperative to improve survival rates of hatchery-reared fish by providing rearing conditions that allow for optimal development of neural and behavioural plasticity, thus producing fish that resemble the "wild type" phenotype more closely.

To improve stocking success, efforts have been directed towards enrichment of the hatchery environment to create more wild-like rearing conditions. These hatchery modifications typically encompass structural enrichment such as rocks, plants and shelter[9]. Structural environmental enrichment (EE) has been shown to improve the learning capacity[10,11], exploratory behaviour[12], prey capture and handling skills[13,14] and, ultimately, post-release survival of hatchery-reared fish in some[15-17], but not all studies[7,18,19]. While the effects of EE on behavioural characteristics of hatchery-reared fish have thus been mapped to some extent, the brain, which underlies these behaviours, remains much understudied. Some studies have demonstrated that EE can increase brain size[20,21], but it is difficult to interpret how a larger brain size relates to neurogenesis and brain plasticity and, ultimately, behaviour[1,22]. Notably, only a few studies report altered expression of neurogenesis markers in the whole telencephalon after EE rearing. For example, Salvanes, et al. [10] reported that EE-rearing increased telencephalic *neurogenic differentiation factor 1* (*neurod*) transcript abundance in Atlantic salmon parr and von Krogh, et al. [23] observed higher numbers of proliferating cell nuclear antigen (Pcna)-expressing cells in EE-reared zebrafish (*Danio rerio*, Hamilton 1822), while Manuel, et al. [24] reported lower telencephalic *neurod* and *pcna* expression in zebrafish reared in an enriched environment. Importantly, the telencephalon consists of a plethora of neuronal subpopulations, each driving distinct behavioural and cognitive processes. For example, the dorsolateral part (Dl) of the telencephalon is involved in relational memory and spatial orientation, while the dorsomedial part (Dm) of the telencephalon is associated with emotional memory and stress reactivity[25-27]. Furthermore, the ventral part of the ventral telencephalon (Vv) is believed to be involved in the regulation of goal-oriented and social behaviour[26,28]. Thus, these regions drive neural processes that may underlie the behaviours which are influenced by EE, and we hypothesise that exposure to EE alters the expression of neuroplasticity and cell proliferation markers in these regions. To date, few studies have investigated the effect of EE on neuroplasticity markers at the level of neuronal subpopulations in fish. Insight in this matter can help

us understand how the rearing environment helps to shape the fish brain, and how this affects the behaviour and, ultimately, post-release survival of hatchery-reared salmonids.

We studied the effects of a seven-week period of EE on telencephalic neuroplasticity and post-release survival success of juvenile Atlantic salmon. Neuroplasticity markers were assessed in the Dl, Dm and Vv subregions of the telencephalon by means of *in situ* hybridisation of the immediate early gene *cfos* and the neurotrophin *brain-derived neurotrophic factor* (*bdnf*), as well as by qPCR of *cfos*, *bdnf*, *pcna* and *neurod* in microdissected tissue from target regions. Post-release survival was assessed by capturing downstream migrating salmon, five months post-release, in a natural river. Our study confirms that EE can increase post-release survival of stocked fish, but we did not find major effects on the expression of the neuroplasticity markers in the selected telencephalic regions after seven weeks of enrichment.

Materials and Methods

Ethical permit

This experiment was performed in accordance with current Norwegian law for experimentation and procedures on live animals, and was approved by the Norwegian Food Safety Authority (Mattilsynet) through FOTS application ID 10034.

Experimental animals

This experiment was conducted at the Norwegian Institute for Nature Research (NINA) salmon hatchery in Ims, using hatchery-reared Atlantic salmon parr (i.e. juvenile salmon that live in fresh water before undergoing smoltification: the metamorphosis that prepares them for their migration into the ocean). The experimental fish which were first generation offspring from wild parents from the river Imsa, south-western Norway (58°50'N; 5°58'E). Fish were transferred to the experimental tanks at 9 months post-hatching and brain sampling and release into the wild took place at 11 months post-hatching. All fish were weighed, measured (total length) and implanted with a passive integrated transponder (PIT) tag prior to the start of the experiment. Early sexually mature (precocious) males were excluded from the study.

Environmental enrichment

In the first week of October 2016, 780 Atlantic salmon parr (152 ± 25 mm total length, 41 ± 18 g body mass; mean ± SD) were randomly distributed between three control and three enriched tanks (n = 130 fish per tank) for a duration of seven weeks. Control tanks were square holding tanks measuring 2×2×1.2 m (l×w×h), filled with approximately 3 m^3 of flow-through ambient river water and covered with a fiberglass lid, through which natural light could penetrate. Enriched tanks were identical to the control tanks, except with the addition of 40 rocks (10 - 20 cm diameter), as well as three artificial plastic 'plants', composed of 8 black fronds (9 cm wide and 100 cm long) each, which were weighted down with a small rock, following methodology described by Salvanes, et al. [10]. Fish appeared to use

both rocks and plants for shelter and fish in the enriched tanks showed less movement and schooling behaviour than control fish in reaction to disturbances, although none of these behavioural responses were quantified. All tanks were manually cleaned every third day by partially draining the water while scrubbing the bottom of the tank with a broom. The rocks and plants in the enriched tanks were rearranged weekly using a net and control tanks were disturbed with the same net for an equal amount of time to control for stress effects. Commercial feed (Nutra Parr, Skretting, Stavanger, Norway) was provided *ad libitum* throughout the day by automatic feeders. Water temperature was 11°C at the start and decreased to 4°C at the end of the experiment. After the enrichment period, all fish were again measured and weighed, and individual specific growth rates (SGR) were calculated as follows:

$$SGR = \left(ln(BM_f) - ln(BM_i)\right) \times \frac{100}{t}$$

where BM_f is the final body mass in g at the end of the enrichment period, BM_i is the initial mass in g at the start of the experiment, and t is the experimental time in days.

Brain sampling

After seven weeks of enrichment, the brains of a subset of fish were sampled, which were processed for quantification of neuroplasticity markers in subregions of the telencephalon by either *in situ* hybridisation (ISH) or qPCR. To this end, fish were randomly collected in pairs from each of the six holding tanks and anaesthetised in 0.75‰ (v/v) 2-phenoxyethanol (Sigma-Aldrich 77699), which rendered them unconscious within 30 sec, after which total length and body mass were recorded. Brains were sampled in two ways: i) to sample brains for ISH, fish were sampled as described by Mes, et al. [3]. In short, anaesthetised fish were fixed by vascular perfusion in 2% paraformaldehyde and brains were then dissected and post-fixed overnight. After three washing steps, brains were cryopreserved overnight in 25% sucrose and subsequently embedded in Tissue-Tek OCT compound, and stored at -80°C until further processing; ii) to sample brains for microdissections and subsequent qPCR analysis, anaesthetised fish were decapitated and the jaw and gills were trimmed away. The tissue was then sealed in a plastic bag, snap-frozen on dry ice and stored at -80°C until processing. Time from decapitation to freezing ranged between 1 and 2 min.

Post-release survival

After the enrichment period, enriched (n = 314) and control (n = 313) PIT-tagged salmon parr were released into the river Imsa during the first week of December to assess their post-release survival and subsequent downstream smolt migration the following spring. To this end, salmon were collected from their tanks, anaesthetised in Benzoak vet (ACD Pharmaceuticals AS; 0.14 ‰ (v/v) in water) and subsequently measured, weighed and adipose fin-clipped for identification, following the standard stocking procedures of the hatchery. Thereafter, fish were allowed to recover for three days in their

holding tanks, but without structural enrichment. The river Imsa is approximately 1 km long and it drains Lake Liavatn into the Høgsfjord[29]. Salmon parr were released at the upstream limit of the river (58.535608, 5.570166), which is marked by a 2-m high barrier, preventing upstream migration of fish into Lake Liavatn. Stocked fish migrated downstream towards the sea in April and May 2017, approximately five months after release. All downstream migrating fish (i.e. both hatchery-reared and indigenous wild fish) were intercepted by a Wolf-type fish trap[30], located 100 m upstream from the estuary. The entire body of water from the river Imsa passes through this trap and therefore every migrating fish is intercepted. The trap was emptied at least twice daily and captured fish were PIT scanned, weighed and measured, after which they were released downstream of the trap so they could resume their ocean migration.

In situ *hybridisation and quantification of labelled cells*

In situ hybridisation for *cfos* and *bdnf* transcripts and subsequent quantification of labelled cells was conducted as described by Mes, et al. [3]. The ISH was performed on transverse parallel sections for eight fish per treatment. Transcript-positive cells were counted in the dorsolateral (both the dorsal and ventral subregions; Dld and Dlv, respectively) and dorsomedial (both the dorsal and ventral subregions; Dmd and Dmv, respectively) pallium, as well as in the ventral part of the ventral telencephalon (Vv; see Mes, et al. [3] for an overview of the subregions). Labelled cells were counted in these five subregions (in both telencephalic lobes) in 11.2 ± 2.8 (mean ± SD) telencephalon sections per fish. Because the number of brain sections differed per individual, we corrected for the number of counted sections by calculating the average number of labelled cells per section for each subregion. These average numbers of labelled cells per section in each area were used in the statistical analysis.

Microdissections

Frozen trimmed skulls of eight fish per treatment were sectioned (100 µm thick) transversely in a cryostat (Leica CM 3050) at -22°C. Sections were thaw-mounted onto glass slides (VWR 631-151) and subsequently stored at -80°C. Microdissections of the Dld, Dlv, Dmd, Dmv and Vv were performed on frozen sections kept on a cooling plate (-14 ‘°C) as described by Vindas, et al. [31]. On average, per individual, a total of 37, 37, 38, 36 and 13 punches were taken for the Dld, Dlv, Dmd, 36 Dmv and Vv, respectively. Microdissected tissue was injected into RLT buffer (RNeasy Micro Kit, Qiagen 74004) and immediately frozen at -80°C until RNA extraction, which was conducted within three days after microdissection.

Relative transcript abundance

Relative transcript abundance of *cfos, bdnf*, *neurod* and *pcna* in microdissected areas was measured using real-time PCR (qPCR). Microdissected tissue was thawed, vortexed for 30 s, centrifuged at 13,400 × *g* for 5 min and total RNA was subsequently extracted using the RNeasy Micro Kit (Qiagen, ID 74004), which includes a DNase I treatment, according to the manufacturer’s instructions. RNA

concentrations were measured using a BioTek Epoch microplate spectrophotometer and the quality of extracted RNA was checked on a subset of samples using a Bioanalyzer RNA 6000 Pico Kit (Agilent 2100): RNA integrity numbers (RIN) were 9.8 ± 0.3 (mean ± SD) with all RIN values above 8.9. Reverse transcription was performed using an iScript cDNA Synthesis Kit (Bio-Rad 1708891) according to the manufacturer's instructions, using 36 ng of total RNA as template in a total reaction volume of 20 µl. Subsequently, cDNA was stored at -20°C.

The four target genes, as well as three reference genes (*elongation factor 1αa* (*ef1αa*), *ribosomal protein S20* (*S20*), and *hypoxanthine phosphoribosyltransferase 1 (hprt1)*) were selected for qPCR (**Table 1**). Previously published primer sequences were available for all genes, except for *cfos*, for which primers were designed in this study. The predicted sequence for *cfos* in Atlantic salmon (accession number: XM_014206157.1) was retrieved from the National Center for Biotechnology Information (NCBI: http://ncbi.nlm.nih.gov/nuccore) and primers were designed using the NCBI Primer-BLAST function. Two *cfos* primer pairs were designed at exon-exon junctions and the primer pair with the lowest Cq values and with a single melting peak was selected for further use (**Table 1**). Calibration curves were run for all primer pairs (**Supplementary Table 1**) and qPCR products were sequenced to confirm the specificity of the primers. The stability of the three reference genes *ef1αa*, *S20* and *hprt1* was evaluated using the NormFinder[32] and geNorm[33] methods, after which *ef1αa* and *S20* were selected as most stable reference genes.

Real-time PCR was carried out in duplicate using a Roche Light Cycler 96 (Roche Diagnostics, Penzberg, Germany) and accompanying software (version 1.1.0.1320). The reaction volume was 10 µl including 5 µl LightCycler® 480 SYBR® Green I Master (04887352001, Roche Diagnostics GmbH, Mannheim, Germany), 1 µl of each forward and reverse primer (1 nM final concentration for each primer) and 3 µl of cDNA (diluted 1:5). Cycling conditions were 10 min at 95°C, followed by 40 cycles of 10 s at 95°C, 10 s at 60°C and 8 s at 72°C, followed by a melting curve analysis. Expression of *neurod* in the Vv was very low (Cq values > 35) and this analysis was therefore excluded, while Cq values of all other genes in all other brain areas were < 35 and thus included in the analysis following methodology of qPCR analysis by Bustin, et al. [34]. A calibrator, made by pooling aliquots of cDNA of all samples, was included in triplicate in all plates to allow for comparison of Cq values between plates. Expression values were computed according to Vandesompele, et al. [33], and expression values are expressed as relative to the expression of the two reference genes (*ef1αa* and *S20*).

Statistical analyses

All data were analysed in JMP Pro 14.0.0 (SAS Institute Inc.) and unless otherwise stated, all values are given as mean ± SEM. A Generalised Linear Model (GLM) with binomial distribution was employed to compare migration success between control and enriched fish. Body size and treatment, as well as an interaction between these two variables, were considered as explanatory variables. The most

parsimonious model (with the lowest corrected Akaike Information Criterion (AICc) score) was a model with only treatment (i.e. control vs. enriched) as explanatory variable, and this model was subsequently used to compare migration success between treatments.

For morphometric data, normality and homogeneity of variance were assessed by Shapiro-Wilk's and Levene's tests. Because the data showed a bimodal distribution, Wilcoxon rank-sum tests were used to compare total length, body mass and SGR between control and enriched groups. To test whether there were differences in body mass between wild and hatchery-reared fish at time of migration, an analysis of covariance (ANCOVA) was used, with body mass as dependent variable, fish origin (hatchery (pooled enriched and control) vs. wild) as independent variable and migration date as a continuous covariate.

In situ hybridisation cell counts were compared using Student's *t*-tests assuming equal variances (homogeneity of variance was verified using Levene's tests). Cell counts of one fish from the enriched treatment were excluded because slides were of insufficient quality. Cell counts in the brain areas of the left and right telencephalic lobes were pooled after assessing (by means of a Spearman's correlation test) that there were no significant lateralisation differences, with the exception of the cell counts of *cfos* within the Dlv. The fact that the number of *cfos* cells in the Dlv between the right and left lobe were not correlated is likely to be due to the low number of expressed cells in this area (0.45 cells per section). Because the number of labelled cells showed a significant bilateral correlation in all other brain areas in the current study, as well as for *cfos* in the Dlv in our previous work[3], *cfos* cell counts in the left and right lobe of the Dlv were also pooled together. To adhere to Gaussian distribution, values were ^{10}log-transformed for *bdnf* (only in the Dlv). For qPCR data, gene expression levels of *cfos*, *bdnf*, *neurod* and *pcna* were compared between control and enriched groups using Wilcoxon rank-sum tests.

Results

Growth during enrichment

At the start of the experiment, control fish measured 150.7 ± 1.4 mm total length and weighed 40.4 ± 1.0 g. Enriched fish measured 153.6 ± 1.2 mm and weighed 41.9 ± 0.9 g, which was not significantly different from control fish (Wilcoxon test: W_s = 129997, Z = -1.20, p = 0.23 and W_s = 130030, Z = -1.18, p = 0.24 for length and body mass, respectively). During the experiment, fish increased on average in body length and decreased in body mass: after seven weeks of enrichment, control fish were 155.0 ± 1.4 mm and 38.2 ± 0.9 g, compared to 158.5 ± 1.2 mm and 39.9 ± 0.8 g for enriched fish, with enriched and control groups not showing any statistically significant differences (W_s = 128977, Z = -1.55 p = 0.12 and W_s = 129173, Z = -1.48, p = 0.14 for length and body mass, respectively). Specific growth rates in the hatchery were negative, and significantly lower for control fish (-0.089 ± 0.007), compared to enriched fish (-0.081 ± 0.006; W_s = 128841, Z = -2.02 p = 0.04).

Post-release growth, survival and timing of downstream migration

A significantly higher number of fish from the enriched environment (50 out of 314 released fish: 15.9%) migrated downstream the following spring compared to control fish (33 out of 313: 10.5%, binomial GLM, $p = 0.04$; **Fig. 1A**). In the same spring (2017), a total of 316 wild smolts also migrated downstream in the river Imsa. Timing of downstream migration seemed to differ between wild and hatchery-reared fish (both enriched and control groups), with the wild fish migrating 1.5 weeks later (**Fig. 1B**). That is, while 95% of the wild fish had migrated downstream by July 17th, 95% of the hatchery-raised fish had already migrated downstream by July 7th.

Upon downstream interception of hatchery-reared fish, it was noticeable that released parr in the largest size range (>170 mm length) had lower survival rates compared to the intermediate sized fish (110-169 mm length): large and intermediate sized fish showed migration success rates of 8% (range 6-9%) and 18% (range 16-22%), respectively (**Fig. 2A**). Because no experimental fish were observed to migrate downstream in the following year, and three-year smolts are rare in the river Imsa[29], we assumed that the observed migration rates equated to survival of the hatchery-reared fish.

Only intermediate sized fish, measuring between 120-149 mm at time of release, had increased in body mass at the time of downstream capture following the 5-month period of river residency, compared to their body mass at time of release. In contrast, both smaller (<119 mm) and larger fish (>150 mm) had lost weight during their river residency (**Fig. 2B**). Average specific growth rates of the surviving hatchery fish in the river were -0.103 ± 0.038 and -0.109 ± 0.039 for control and enriched fish, respectively, which were not statistically different from each other (Wilcoxon test, $W_s = 1392$, $Z = 0.05$, $p = 0.96$). Body mass at time of downstream migration was significantly affected by both fish origin (wild *vs.* hatchery; $F = 31(1, 394)$, $p < 0.0001$) and migration date ($F = 106(1, 394)$, $p < 0.0001$), where wild fish had a higher body mass than hatchery-reared fish and body mass decreased during the migration season (**Supplementary Figure 1**).

In situ *hybridisation*

Quantification of *in situ* hybridisation images revealed region-specific expression patterns of *cfos* and *bdnf* in the subregions of the telencephalon (**Fig. 3**). The number of *bdnf* labelled cells was significantly lower in the Dlv of enriched fish compared to controls (Student's *t*-test, $t = -2.08$, $df = 13$, $p = 0.0290$, **Fig. 3B**), while the number of *bdnf*-labelled cells showed a strong tendency for significant differences in the Dld ($t = -1.63$, $df = 13$, $p = 0.0634$) and the Dl as a whole ($t = -1.77$, $df = 13$, $p = 0.0501$). No significant differences in the number of *cfos* or *bdnf*-labelled cells between treatment groups were found in any of the other regions (**Supplementary Table 2**).

Relative transcript abundance

No significant differences in relative mRNA abundance were found between control and enriched fish for *cfos, bdnf, neurod* or *pcna* in the Dld, Dlv, Dmd, Dmv or Vv (**Fig. 4**; **Supplementary Table 3**).

Discussion

In this study, we demonstrate that modifications of the hatchery environment can have profound effects on fish survival in stocking programs. A seven-week exposure to environmental enrichment increased post-release survival of hatchery-reared Atlantic salmon parr by 51% compared to fish reared under standard hatchery conditions. Notably, hatchery-reared stocked fish (both from an enriched environment and controls) of intermediate size classes (110-170 mm) showed higher survival rates than both larger and smaller size classes. Interestingly, after seven weeks of enrichment treatment, no major differences in the expression of the neuroplasticity markers were detected in the selected regions of the telencephalon, as assessed through both ISH and qPCR. This is contrary to previous enrichment studies, which report altered expression of cell proliferation and differentiation markers in the entire forebrain. However, our findings are in agreement with recent studies which propose that telencephalic region-specific gene expression patterns are not always in agreement with whole-telencephalon gene expression patterns [31] and demonstrate that further studies are needed to elucidate the role of the brain in enrichment-induced behavioural plasticity and post-release survival success.

It has been reported that EE can affect a wide range of fish behavioural outputs, including a reduction of conspecific aggression[35,36], an increase of shelter seeking[37,38] and exploration behaviour[24], as well as more favourable post-release habitat selection[17], improved spatial memory[10,11,39] and increased feeding efficiency on novel prey[14,17]. These behaviours are mostly under control of the telencephalon, which contains neural subpopulations associated with processes such as relational and emotional memory, stress coping and goal-oriented behaviour[26,27,40,41]. Recent studies report that EE affects the expression of neurogenesis marker *pcna* and cell differentiation marker *neurod* in the whole telencephalon[10,23,24], which led to our hypothesis that EE alters region-specific expression patterns of neuroplasticity markers in the salmon forebrain. However, in most of the studied neuroplasticity markers, we found no differences in expression between treatments, with the exception of a lower number of *bdnf*-labelled cells in the Dlv of EE-exposed fish. Even though we cannot exactly pinpoint the specific reason why we did not find a difference in the regulation of neuroplasticity markers between enrichment and control groups based on the information that we have obtained in this study, we speculate on several possibilities that should be investigated further. First, the longevity of the exposure to EE can be an important factor, as both neural[24] and behavioural[11] effects of EE have been shown to vary with exposure duration and age of the animals. Second, the telencephalon is comprised of a myriad of neuronal populations with specific functionalities. In response to a stimulus, signalling molecules may be upregulated in one, but downregulated in another region[42]. Importantly, when quantifying gene expression in the entire telencephalon, all regional differences are pooled into one average expression

level, which may not be reflected in the individual areas. It is also possible that differences might occur in subregions not included in the current study, and therefore these differences have escaped detection. However, the Dl and Dm are important drivers of the behavioural parameters that have been reported to be affected by EE, such as spatial memory and emotional decision making[10,35], and are thus the most likely candidate regions to be affected.

Using *in situ* hybridisation, we were able to reveal distinct expression patterns of *cfos* and *bdnf* in the subregions of the telencephalon. Interestingly, in a previous study we found that wild-caught salmon parr show a significantly lower number of *bdnf*-expressing cells in the Dlv, Dmd, Dmv and Vv, compared to hatchery-reared parr with the same genetic background[3]. In this respect, the lower *bdnf* expression seen in the Dlv of enriched fish in the current study resembles the wild phenotype more closely than control fish. We have previously hypothesised that higher *bdnf* expression in the Dlv of hatchery-reared salmon, compared to wild fish, may be linked to stressors in the hatchery environment[3]. In this context, it is tempting to speculate that, as EE has been shown to reduce stress[37], the decrease of *bdnf* expression in the Dlv of enriched fish may be a direct consequence of the stress-alleviating effects of EE. Interestingly, this treatment-associated difference in *bdnf* expression was not corroborated by the qPCR data in microdissected Dlv tissue. Although a contradictory finding at first sight, it is important to consider that quantification of ISH-labelled cells is a binary process (i.e. cells are either counted as expressing or non-expressing and no quantitative measure per cell is included), therefore it is possible that even though there are fewer ISH-labelled *bdnf* cells in the Dlv of enriched animals, these fewer cells could, on average, have a higher abundance of *bdnf* mRNA, masking the effects seen in the ISH analyses (quantified on the cell level) in the qPCR analysis (which quantifies whole-tissue transcript abundance). Alternatively, ISH image analysis allows for precise identification of the entire subregions, while mechanical microdissections with a needle may not be as precise and thus might fail to include all relevant cells within the target neural population, which may underlie the discrepancies found between ISH and qPCR results. To exclude this confounding factor, laser microdissections should be considered for future studies.

The effect of EE on post-release survival of salmonids is inconsistent among studies: while it is positive in some studies[15-17], others find no effects[7,18,19,43], or even a negative effect[35,44]. A problem with comparing enrichment studies is the large variation in methodology, most notably with regards to the type and duration of the enrichment, age of the fish, release date and duration, recapture methods and the characteristics of the studied waterway [9]. Because EE had minor observable effects on neuroplasticity in this study, we can only speculate as to what caused increased post-release survival rates in fish subjected to EE. Environmental enrichment is known to reduce stress responsivity in captivity[37], and stress is known to have adverse effects on cognitive performance[45] and post-release survival[46], which could imply that a higher allostatic load during hatchery-rearing leads to reduced fitness of control individuals. Thus, it is possible that environmental enrichment partly alleviates the

allostatic load on the fish and thus produces a more robust animal, which is better equipped to deal with stressors and changes in the environment. Another interesting observation is that exposure to EE can improve cryptic colouration (i.e. camouflage pattern) of reared fish[16], which is linked to reduced predation susceptibility[47]. Furthermore, colouration is known to play a role in establishing conspecific dominance[48], allowing fish to occupy more favourable habitats. We did, however, not test for these parameters in this study, but suggest that future work should include plasma cortisol analyses at basal and post-handling conditions, as well as a body colouration assessment.

In this study, hatchery fish migrated 1.5 weeks earlier than wild fish, and while enrichment increased post-release survival, it did not alter the timing of downstream migration. Asynchrony in migration timing between wild and hatchery salmonids has been reported previously, in some cases with hatchery-reared fish migrating earlier[49], or later[50,51] than their wild conspecifics. The implications of sub-optimal migration timing may be particularly severe for long-river populations, where late-migrating fish run the risk of missing their physiological smolt window upon arrival at the ocean[52], and may therefore not be able to survive in seawater. Additionally, early marine feeding is an important driver of smolt survival and sub-optimal smolt migration timing may lead to a mismatch with peak marine prey abundance[53]. Interestingly, several studies report increased post-release survival with increased body size at time of release in hatchery-reared salmon[35,54]. Although in this study, the smallest released parr did not survive in the wild, survival rates of the largest parr (>170 mm) were noticeably lower than those of intermediate sized parr (110-170 mm). A possible explanation for the lower survival rates of the largest stocked parr could be related to starvation effects, as we observed that fish of larger size classes were unable to maintain their body mass after release into the wild, indicating insufficient food acquisition. Although supporting evidence is lacking, it is also possible that larger parr are more vulnerable to predator-related mortality. It is a common stocking practice to release fish of large size to increase survival probability: a strategy which seems effective when salmonids are released as smolts directly before migration[15,35,44]. However, when fish will remain in the river for a longer period before embarking on their migration, our data suggest that stocked fish should be of intermediate size at time of release. Finally, as we observed that large fish migrate earlier than smaller fish, selection and stocking of intermediate-sized parr might also reduce the difference in migration timing between wild and stocked individuals.

Few studies on teleosts use an experimental approach to determine the optimal conditions of EE, but there are indications that the duration of exposure to EE[11,24], age of the fish[24] and the type of enrichment (in mice[55]) can affect the efficacy of EE in altering animal behaviour and neuroplasticity. Further empirical testing of optimal EE conditions can shed light on inconsistencies between current enrichment studies and contribute to optimisation of hatchery protocols. As hatchery managers are hesitant to implement EE because of hygiene and increased labour concerns, it is also important to investigate alternative innovative hatchery protocols to structural enrichment. For example, reduced rearing density

has been shown to improve post-release survival of salmonids[43,56], while environmental variability and unpredictability promotes behavioural flexibility in Atlantic cod (*Gadus morhua* L.)[57] and predator conditioning using visual and/or olfactory cues can improve predator avoidance[58,59]. Additionally, swimming exercise could potentially be an effective method to improve the behavioural responses and post-release performance of fish, as mammalian studies indicate that exercise, not structural environmental enrichment, is the main driver of environmentally-induced neurogenesis and neuroplasticity[60,61], and several studies report increased post-release survival for exercised salmonids compared to sedentary individuals[62,63].

In conclusion, we here report increased post-stocking success for EE-reared fish compared to control fish, while we only find minimal regional changes in forebrain neural plasticity marker expression. We suggest that future studies should empirically elucidate the optimal conditions of EE and compare its efficacy to other innovative hatchery protocols. Even though the neural mechanisms have not been entirely uncovered, our results suggest that future Atlantic salmon hatchery strategies should provide EE and aim to produce or select intermediate size classes of fish for stocking, to improve the post-release survival of stocked Atlantic salmon parr in short rivers.

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Author Contributions

D.M., M.G., L.O.E.E., B.F., I.M. and M.A.V. contributed conception and design of the study; D.M. and R.v.O. collected the samples and performed laboratory analyses; D.M., R.v.O., M.G. and M.A.V. conducted statistical analysis; D.M. and M.A.V. wrote the first draft of the manuscript. All authors contributed significantly to manuscript revision and approved the final version.

Competing Interests

The authors declare no competing interests.

Data Availability

All data generated or analysed during this study are included in this published article and its Supplementary Information files.

References

1 Johnsson, J. I., Brockmark, S. & Näslund, J. Environmental effects on behavioural development consequences for fitness of captive-reared fishes in the wild. *J. Fish Biol.* **85**, 1946-1971, doi:10.1111/jfb.12547 (2014).

2 Olla, B. L., Davis, M. W. & Ryer, C. H. Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bull. Mar. Sci.* **62**, 531-550 (1998).

3 Mes, D., Von Krogh, K., Gorissen, M., Mayer, I. & Vindas, M. A. Neurobiology of wild and hatchery-reared Atlantic salmon: How nurture drives neuroplasticity. *Front. Behav. Neurosci.*, doi:10.3389/fnbeh.2018.00210 (2018).

4 Palmé, A., Wennerstöm, L., Guban, P., Ryman, N. & Laikre, L. Compromising Baltic salmon genetic diversity - conservation genetic risks assiciated with compensatory releases of salmon in the Balctic Sea. *Havs- och vattenmyndighetens rapport 2012:18*, 115 pp (2012).

5 Finstad, B. & Jonsson, N. Factors influencing the yield of smolt releases in Norway. *Nord. J. Freshwat. Res.* **75**, 37-55 (2001).

6 Jonsson, N., Jonsson, B. & Hansen, L. P. The marine survival and growth of wild and hatchery-reared Atlantic salmon. *J. Appl. Ecol.* **40**, 900-911, doi:10.1046/j.1365-2664.2003.00851.x (2003).

7 Tatara, C. P., Riley, S. C. & Scheurer, J. A. Growth, survival, and habitat use of naturally reared and hatchery steelhead fry in streams: Effects of an enriched hatchery rearing environment. *Trans. Am. Fish. Soc.* **138**, 441-457, doi:10.1577/t07-260.1 (2009).

8 Thorstad, E. B., Uglem, I., Arechavala-Lopez, P., Økland, F. & Finstad, B. Low survival of hatchery-released Atlantic salmon smolts during initial river and fjord migration. *Boreal Environ. Res.* **16**, 115-120 (2011).

9 Näslund, J. & Johnsson, J. I. Environmental enrichment for fish in captive environments: effects of physical structures and substrates. *Fish Fish.* **17**, 1-30, doi:10.1111/faf.12088 (2014).

10 Salvanes, A. G. *et al.* Environmental enrichment promotes neural plasticity and cognitive ability in fish. *Proc. R. Soc. B* **280**, 20131331, doi:10.1098/rspb.2013.1331 (2013).

11 Ahlbeck Bergendahl, I., Salvanes, A. G. V. & Braithwaite, V. A. Determining the effects of duration and recency of exposure to environmental enrichment. *Appl. Anim. Behav. Sci.* **176**, 163-169, doi:10.1016/j.applanim.2015.11.002 (2016).

12 Lee, J. S. F. & Berejikian, B. A. Effects of the rearing environment on average behaviour and behavioural variation in steelhead. *J. Fish Biol.* **72**, 1736-1749, doi:10.1111/j.1095-8649.2008.01848.x (2008).

13 Sundström, L. F. & Johnsson, J. I. Experience and social environment influence the ability of young brown trout to forage on live novel prey. *Anim. Behav.* **61**, 249-255, doi:10.1006/anbe.2000.1593 (2001).

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

15 Hyvärinen, P., Rodewald, P. & Bradford, M. Enriched rearing improves survival of hatchery-reared Atlantic salmon smolts during migration in the river Tornionjoki. *Can. J. Fish. Aquat. Sci.* **70**, 1386-1395, doi:10.1139/cjfas-2013-0147 (2013).

16 Maynard, D. J., Flagg, T. A. & Mahnken, C. V. W. in *Am. Fish. Soc. Symp.* Vol. 15 (1995).

17 Roberts, L. J., Taylor, J., Gough, P. J., Forman, D. W. & Garcia de Leaniz, C. Silver spoons in the rough: can environmental enrichment improve survival of hatchery Atlantic salmon *Salmo salar* in the wild? *J. Fish Biol.* **85**, 1972-1991, doi:10.1111/jfb.12544 (2014).

18 Brockmark, S., Neregård, L., Bohlin, T., Björnsson, B. T. & Johnsson, J. I. Effects of rearing density and structural complexity on the pre- and postrelease performance of Atlantic salmon. *Trans. Am. Fish. Soc.* **136**, 1453-1462, doi:10.1577/t06-245.1 (2007).

19 Fast, D. E. *et al.* Survival comparison of spring Chinook salmon reared in a production hatchery under optimum conventional and seminatural conditions. *Trans. Am. Fish. Soc.* **137**, 1507-1518, doi:10.1577/t07-143.1 (2008).

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

21 Näslund, J., Aarestrup, K., Thomassen, Søren T., Johnsson, Jörgen I. & Post, J. Early enrichment effects on brain development in hatchery-reared Atlantic salmon (*Salmo salar*): no evidence for a critical period. *Can. J. Fish. Aquat. Sci.* **69**, 1481-1490, doi:10.1139/f2012-074 (2012).

22 Ebbesson, L. O. & Braithwaite, V. A. Environmental effects on fish neural plasticity and cognition. *J. Fish Biol.* **81**, 2151-2174, doi:10.1111/j.1095-8649.2012.03486.x (2012).

23 von Krogh, K., Sorensen, C., Nilsson, G. E. & Overli, O. Forebrain cell proliferation, behavior, and physiology of zebrafish, *Danio rerio*, kept in enriched or barren environments. *Physiol. Behav.* **101**, 32-39, doi:10.1016/j.physbeh.2010.04.003 (2010).

24 Manuel, R. *et al.* The effects of environmental enrichment and age-related differences on inhibitory avoidance in zebrafish (*Danio rerio* Hamilton). *Zebrafish* **12**, 152-165, doi:10.1089/zeb.2014.1045 (2015).

25 Portavella, M., Torres, B. & Salas, C. Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. *J. Neurosci.* **24**, 2335-2342, doi:10.1523/jneurosci.4930-03.2004 (2004).

26 O'Connell, L. A. & Hofmann, H. A. The vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *J. Comp. Neurol.* **519**, 3599-3639, doi:10.1002/cne.22735 (2011).

27 Broglio, C. *et al.* Hippocampal pallium and map-like memories through vertebrate evolution. *J. Behav. Brain. Sci* **5**, 109, doi:10.4236/jbbs.2015.53011 (2015).

28 Vargas, J. P., López, J. C. & Portavella, M. What are the functions of fish brain pallium? *Brain Res. Bull.* **79**, 436-440, doi:10.1016/j.brainresbull.2009.05.008 (2009).

29 Jonsson, N., Jonsson, B. & Hansen, L. P. Long-term study of the ecology of wild Atlantic salmon smolts in a small Norwegian river. *J. Fish Biol.* **52**, 638-650, doi:10.1111/j.1095-8649.1998.tb02023.x (1998).

30 Wolf, P. A trap for the capture of fish and other organisms moving downstream. *Trans. Am. Fish. Soc.* **80**, 41-45, doi:10.1577/1548-8659(1950)80[41:ATFTCO]2.0.CO;2 (1951).

31 Vindas, M. A. *et al.* How do individuals cope with stress? Behavioural, physiological and neuronal differences between proactive and reactive coping styles in fish. *J. Exp. Biol.*, 1524-1532, doi:10.1242/jeb.153213 (2017).

32 Andersen, C. L., Jensen, J. L. & Ørntoft, T. F. Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res.* **64**, 5245, doi:10.1158/0008-5472.CAN-04-0496 (2004).

33 Vandesompele, J. *et al.* Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* **3**, 1-12, doi:10.1186/gb-2002-3-7-research0034 (2002).

34 Bustin, S. A. *et al.* The MIQE guidelines: *M*inimum *I*nformation for publication of *Q*uantitative real-time PCR *E*xperiments. *Clin. Chem.* **55**, 611, doi:10.1373/clinchem.2008.112797 (2009).

35 Rosengren, M., Kvingedal, E., Näslund, J., Johnsson, J. I. & Sundell, K. Born to be wild: effects of rearing density and environmental enrichment on stress, welfare, and smolt migration in hatchery-reared Atlantic salmon. *Can. J. Fish. Aquat. Sci.* **74**, 1-10, doi:10.1139/cjfas-2015-0515 (2016).

36 Berejikian, B. A. Rearing in enriched hatchery tanks improves dorsal fin quality of juvenile steelhead. *N. Am. J. Aquacult.* **67**, 289-293, doi:10.1577/A05-002.1 (2005).

37 Näslund, J. *et al.* Hatchery tank enrichment affects cortisol levels and shelter-seeking in Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **70**, 585-590, doi:10.1139/cjfas-2012-0302 (2013).

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

39 Roy, T. & Bhat, A. Learning and memory in juvenile zebrafish: What makes the difference – population or rearing environment? *Ethology* **122**, 308-318, doi:10.1111/eth.12470 (2016).

40 Broglio, C. *et al.* Hallmarks of a common forebrain vertebrate plan: Specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. *Brain Res. Bull.* **66**, 277-281, doi:10.1016/j.brainresbull.2005.03.021 (2005).

41 Rodríguez, F., Broglio, C., Durán, E., Gómez, A. & Salas, C. in *Fish Cognition and Behavior* (eds C. Brown, K. Laland, & J. Krause) (2007).

42 Summers, C. H. & Winberg, S. Interactions between the neural regulation of stress and aggression. *J. Exp. Biol.* **209**, 4581, doi:10.1242/jeb.02565 (2006).

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

44 Berejikian, B. A., Smith, R. J. F., Tezak, E. P., Schroder, S. L. & Knudsen, C. M. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. *Can. J. Fish. Aquat. Sci.* **56**, 830-838, doi:10.1139/f99-010 (1999).

45 Gaikwad, S. *et al.* Acute stress disrupts performance of zebrafish in the cued and spatial memory tests: The utility of fish models to study stress–memory interplay. *Behav. Process.* **87**, 224-230, doi:10.1016/j.beproc.2011.04.004 (2011).

46 Teixeira, C. P., de Azevedo, C. S., Mendl, M., Cipreste, C. F. & Young, R. J. Revisiting translocation and reintroduction programmes: the importance of considering stress. *Anim. Behav.* **73**, 1-13, doi:10.1016/j.anbehav.2006.06.002 (2007).

47 Donnelly, W. A. & Whoriskey, F. G. in *Production of juvenile Atlantic salmon, Salmo salar, in natural waters* (eds R.J. Gibson & R.E. Cutting) 25-34 (Can. Spec. Publ. Fish. Aquat. Sci. 118, 1993).

48 Höglund, E., Balm, P. H. & Winberg, S. Skin darkening, a potential social signal in subordinate arctic charr (*Salvelinus alpinus*): The regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. *J. Exp. Biol.* **203**, 1711-1721 (2000).

49 Stich, D. S., Zydlewski, G. B., Kocik, J. F. & Zydlewski, J. D. Linking behavior, physiology, and survival of Atlantic salmon smolts during estuary migration. *Marine and Coastal Fisheries* **7**, 68-86, doi:10.1080/19425120.2015.1007185 (2015).

50 Urke, H. A., Kristensen, T., Ulvund, J. B. & Alfredsen, J. A. Riverine and fjord migration of wild and hatchery-reared Atlantic salmon smolts. *Fish. Manage. Ecol.* **20**, 544-552, doi:10.1111/fme.12042 (2013).

51 Chittenden, C. M. *et al.* Riverine, estuarine and marine migratory behaviour and physiology of wild and hatchery-reared coho salmon *Oncorhynchus kisutch* (Walbaum) smolts descending the Campbell River, BC, Canada. *J. Fish Biol.* **72**, 614-628, doi:10.1111/j.1095-8649.2007.01729.x (2008).

52 McCormick, S. D., Hansen, L. P., Quinn, T. P. & Saunders, R. L. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **55**, 77-92, doi:10.1139/d98-011 (1998).

53 Thorstad, E. B. *et al.* A critical life stage of the Atlantic salmon *Salmo salar*: behaviour and survival during the smolt and initial post-smolt migration. *J. Fish Biol.* **81**, 500-542, doi:10.1111/j.1095-8649.2012.03370.x (2012).

54 Kallio-Nyberg, I., Jutila, E., Saloniemi, I. & Jokikokko, E. Association between environmental factors, smolt size and the survival of wild and reared Atlantic salmon from the Simojoki River in the Baltic Sea. *J. Fish Biol.* **65**, 122-134, doi:10.1111/j.0022-1112.2004.00435.x (2004).

55 Lambert, T. J., Fernandez, S. M. & Frick, K. M. Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. *Neurobiol. Learn. Mem.* **83**, 206-216, doi:10.1016/j.nlm.2004.12.001 (2005).

56 Larsen, M. H., Johnsson, J. I., Näslund, J., Thomassen, S. T. & Aarestrup, K. Reduced rearing density increases postrelease migration success of Atlantic salmon (*Salmo salar*) smolts. *Can. J. Fish. Aquat. Sci.* **73**, 804-810, doi:10.1139/cjfas-2014-0563 (2016).

57 Braithwaite, V. A. & Salvanes, A. G. V. Environmental variability in the early rearing environment generates behaviourally flexible cod: implications for rehabilitating wild populations. *Proc. R. Soc. B* **272**, 1107, doi:10.1098/rspb.2005.3062 (2005).

58 Berejikian, B. A., Tezak, E. P. & LaRae, A. L. Innate and enhanced predator recognition in hatchery-reared chinook salmon. *Environ. Biol. Fishes* **67**, 241-251, doi:10.1023/A:1025887015436 (2003).

59 D'Anna, G. *et al.* Effects of predator and shelter conditioning on hatchery-reared white seabream *Diplodus sargus* (L., 1758) released at sea. *Aquaculture* **356-357**, 91-97, doi:10.1016/j.aquaculture.2012.05.032 (2012).

60 van Praag, H., Kempermann, G. & Gage, F. H. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* **2**, 266-270 (1999).

61 Voss, M. W., Vivar, C., Kramer, A. F. & van Praag, H. Bridging animal and human models of exercise-induced brain plasticity. *Trends Cogn. Sci.* **17**, 525-544, doi:10.1016/j.tics.2013.08.001 (2013).

62 Cresswell, R. C. & Williams, R. Post- stocking movements and recapture of hatchery-reared trout released into flowing waters - effect of prior acclimation to flow. *J. Fish Biol.* **23**, 265-276, doi:10.1111/j.1095-8649.1983.tb02904.x (1983).

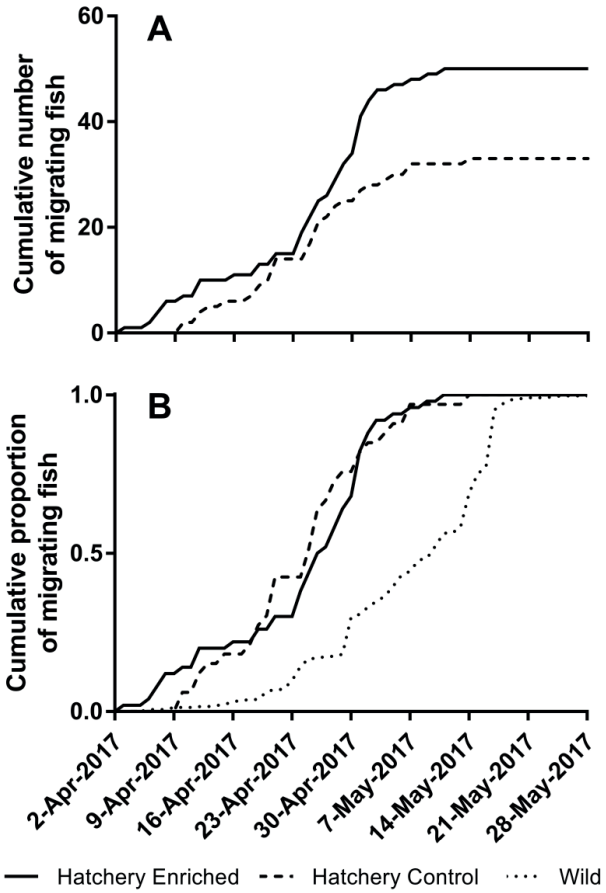
63 Burrows, R. E. The influence of fingerling quality on adult salmon survivals. *Trans. Am. Fish. Soc.* **98**, 777-784, doi:10.1577/1548-8659(1969)98[777:TIOFQO]2.0.CO;2 (1969).

64 Ingerslev, H.-C., Pettersen, E. F., Jakobsen, R. A., Petersen, C. B. & Wergeland, H. I. Expression profiling and validation of reference gene candidates in immune relevant tissues and cells from Atlantic salmon (*Salmo salar* L.). *Mol. Immunol.* **43**, 1194-1201, doi:10.1016/j.molimm.2005.07.009 (2006).

65 Olsvik, P. A., Lie, K. K., Jordal, A.-E. O., Nilsen, T. O. & Hordvik, I. Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. *BMC Mol. Biol.* **6**, 21, doi:10.1186/1471-2199-6-21 (2005).

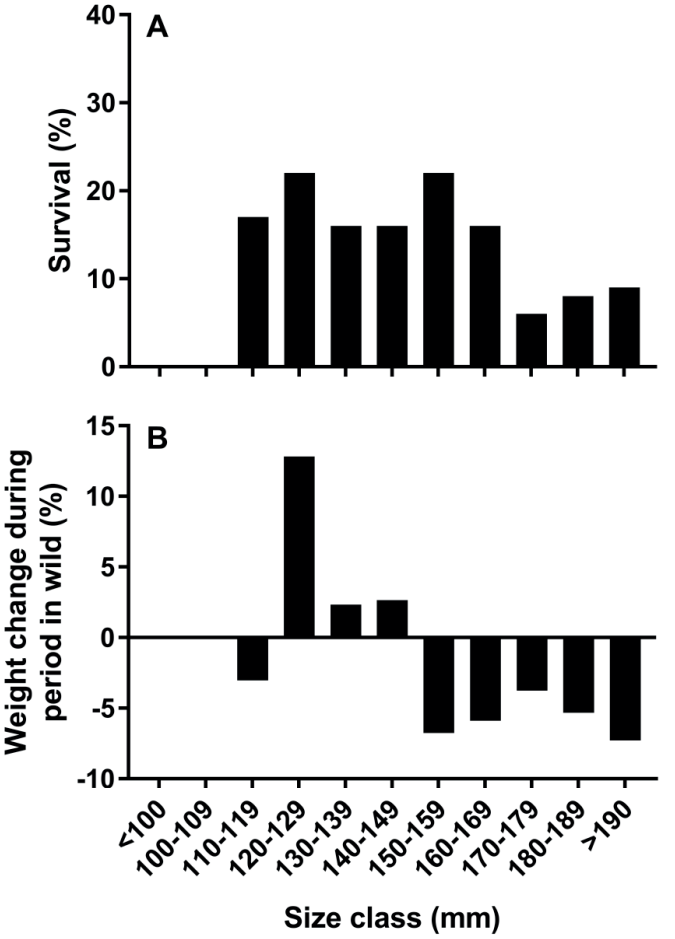
66 Andreassen, R., Lunner, S. & Høyheim, B. Characterization of full-length sequenced cDNA inserts (FLIcs) from Atlantic salmon (*Salmo salar*). *BMC Genomics* **10**, 502-502, doi:10.1186/1471-2164-10-502 (2009).

67 Vindas, M. A. *et al.* Coping with unpredictability: dopaminergic and neurotrophic responses to omission of expected reward in Atlantic salmon (*Salmo salar* L.). *PloS one* **9**, e85543, doi:10.1371/journal.pone.0085543 (2014).



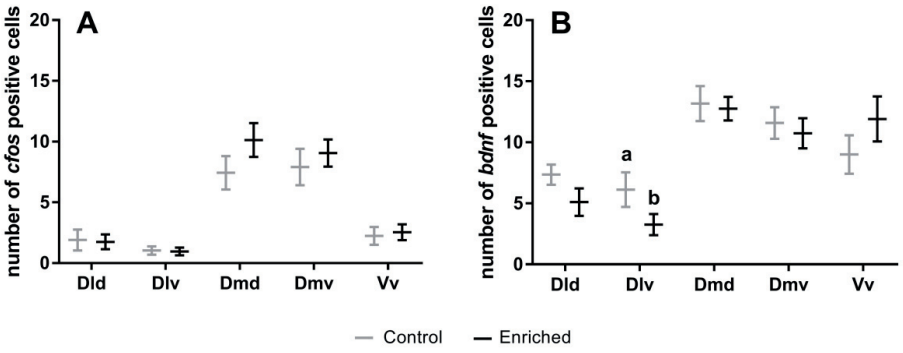
605

606 **Figure 1. Migration of hatchery-reared and wild Atlantic salmon.** After five months in the wild, a
607 significant (p = 0.04) higher number of EE-reared hatchery fish (50 out of 314 released individuals)
608 completed downstream migration compared to control fish (33 out of 313; **A**). Native wild fish (n =
609 316) migrated approximately 1.5 week later than both enriched and control hatchery-reared fish (**B**).



610

611 **Figure 2. Intermediate sized stocked fish show highest post-release survival and growth rates.**
612 Stocked hatchery-reared Atlantic salmon (grouped data for control and enriched fish) with a total length
613 >170 mm show reduced survival compared to intermediate sized fish and fish <110 mm show no
614 survival during a five-month winter in the wild (**A**). Only hatchery-reared fish between 120-149 mm
615 increase in body mass during river residency, while larger and smaller size classes lose weight in the
616 wild (**B**). Total number of released fish per size class: <100 mm: $n = 11$; 100-109 mm: $n = 21$; 110-119
617 mm: $n = 24$; 120-129 mm: $n = 18$; 130-139 mm: $n = 19$; 140-149 mm: $n = 51$, 150-159: $n = 113$; 160-
618 169 mm: $n = 144$; 170-179 mm: $n = 124$, 180-189 mm: $n = 69$; >190 mm: $n = 33$.



619

620 **Figure 3. *In situ* hybridisation labelled *cfos* and *bdnf* cells in telencephalon subregions.** Mean ±
621 SEM number of *cfos* (**A**) and *bdnf* (**B**) positive cells in the dorsal (Dld) and ventral (Dlv) dorsolateral
622 telencephalon, the dorsal (Dmd) and ventral (Dmv) dorsomedial telencephalon, and in the ventral part
623 of the ventral telencephalon (Vv) in Atlantic salmon parr after seven weeks in a control (grey bars) or
624 enriched environment (black bars). Lowercase letters symbolise significant differences between
625 treatment groups (Student's *t*-test, $p = 0.03$). For control animals, $n = 8$ per brain area, while for enriched
626 animals, $n = 7$ per area.

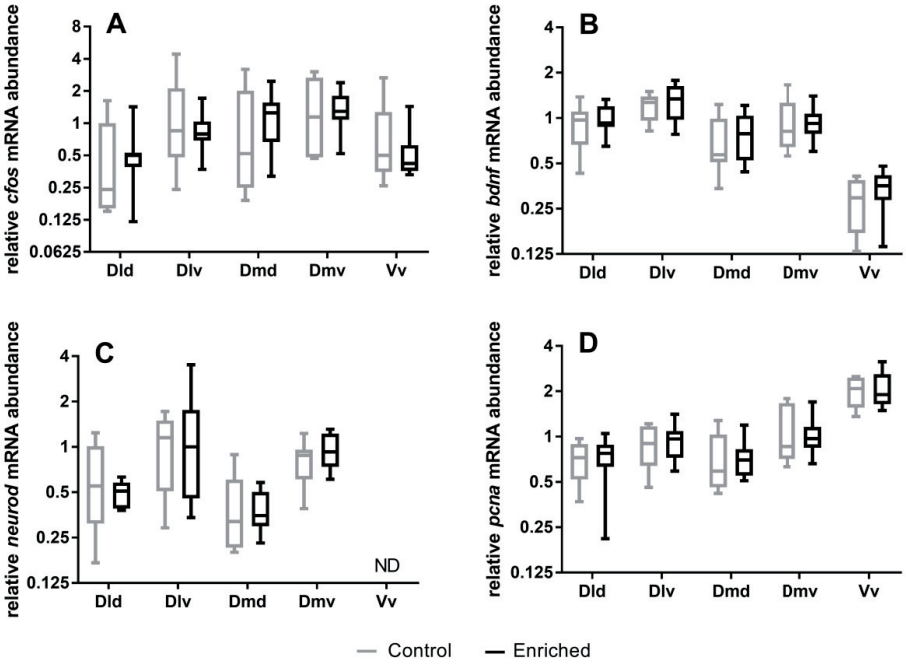


Figure 4. Effect of environmental enrichment on expression of neuroplasticity markers in telencephalon subregions. Box plots depicting relative transcript abundance of *cfos* (**A**), *bdnf* (**B**), *neurod* (**C**) and *pcna* (**D**) in five microdissected areas of the telencephalon: the dorsal (Dld) and ventral (Dlv) part of the dorsolateral telencephalon, the dorsal (Dmd) and ventral (Dmv) part of the dorsomedial telencephalon, and in the ventral part of the ventral telencephalon (Vv). Center lines within the box plots represent the median, boxes reflect quartiles, and whiskers depict minimum and maximum expression values. In the Vv, *neurod* was not detected (ND). Transcript abundance is relative to expression of reference genes *ef1αa* and *S20*, $n = 8$ per brain area.

637 **Table 1. Primer sequences for target genes.**

Gene	Primer Sequence 5' → 3'	Accession no.	Reference
ef1αa	Fw CCCCTCCAGGACGTTTACAAA Rev CACACGGCCCACAGGTACA	BT059133.1	Ingerslev, et al. [64]
S20	Fwd GCAGACCTTATCCGTGGAGCTA Rev TGGTGATGCGCAGAGTCTTG	NM_001140843.1	Olsvik, et al. [65]
hprt1	Fwd CGTGGCTCTCTGCGTGCTCA Rev TGGAGCGGTCGCTGTTACGG	BT043501.1	Andreassen, et al. [66]
bdnf	Fwd ATGTCTGGGCAGACCGTTAC Rev GTTGTCCTGCATTGGGAGTT	GU108576.1	Vindas, et al. [67]
cfos	Fwd AATGGAACAGCTTTCGCCTGA Rev TGTCGGTGAGTTCCTTTCGC	XM_014206157.1	This study
pcna	Fwd TGAGCTCGTCGGGTATCTCT Rev CTCGAAGACTAGGGCGAGTG	BT056931.1	Vindas, et al. [67]
neurod	Fwd CAATGGACAGCTCCCACATCT Rev CCAGCGCACTTCCGTATGA	BT058820.1	Vindas, et al. [67]

638

Effects of environmental enrichment on forebrain neural plasticity and survival success of stocked Atlantic salmon

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Supplementary Material

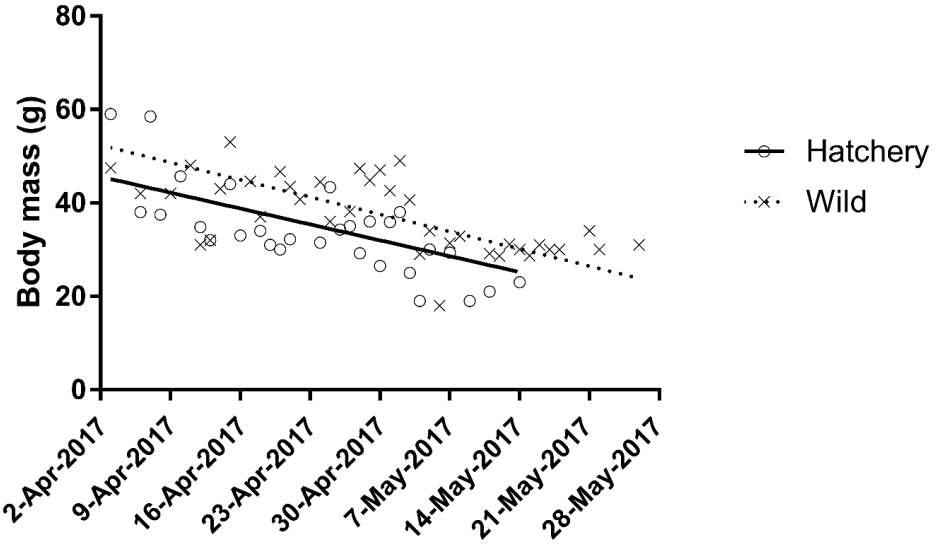

Figure S1. Larger fish migrate earlier than smaller fish. Larger smolts migrated earlier in the season than smaller smolts (ANCOVA, $F = 106(1, 394)$, $p < 0.0001$) and wild fish (circles) had a higher body mass than hatchery-reared fish (crosses; ANCOVA; $F = 31(1, 394)$, $p < 0.0001$)

Table S1. qPCR validation.

	s20	*ef1αa*	*bdnf*	*cfos*	*pcna*	*neurod*
Slope	-3.357	-3.2397	-3.2745	-3.3122	-3.1547	-3.3124
Efficiency	1.99	2.04	2.02	2	2.07	2
Error	0.21	0.13	0.36	0.34	0.2	0.33
R^2	0.99	1	0.99	0.99	0.99	0.98
Y-Intercept	21.58	22.52	24.42	27.61	26.56	27.59

Table S2. *In situ* hybridisation statistics (Student's *t* tests). For all tests, *df* = 13.

	cfos		*bdnf*	
Brain region	*t*-statistic	*p*-value	*t*-statistic	*p*-value
Dld	0.07	0.53	-1.63	0.063
Dlv	0.32	0.62	-2.08	**0.029**
Dmd	1.36	0.90	-0.23	0.41
Dmv	0.60	0.72	-0.47	0.33
Vv	0.30	0.62	1.21	0.88

Table S3. qPCR statistics (Wilcoxon tests).

	cfos			*bdnf*			*neurod*			*pcna*		
Brain region	W_s	Z	p-value	W_s	Z	p-value	W_s	Z	p-value	W_s	Z	p-value
Dld	54	-0.17	0.86	73	0.47	0.63	49	-0.38	0.70	70	0.16	0.87
Dlv	58	0.17	0.86	75	0.68	0.49	70	0.16	0.87	55	-0.06	0.95
Dmd	52	-0.4	0.69	53	-0.35	0.73	71	0.26	0.79	56	0.00	1.00
Dmv	70	0.16	0.87	75	0.63	0.53	48	-0.93	0.35	71	0.26	0.79
Vv	60	0.35	0.73	77	0.84	0.40	N/A	N/A	N/A	69	0.05	0.96

III

Brain training: can swimming exercise enhance brain plasticity and cognition in Atlantic salmon?

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Abstract

It is well-established that running exercise can enhance brain plasticity and boost cognitive performance in mammals, but this phenomenon has not received much attention in fish. The aim of this study was to determine whether an 8-week sustained swimming exercise regime can enhance brain plasticity and cognition in juvenile Atlantic salmon. Fish were subjected to either a fixed (forced exercise) or variable (volitional exercise) flow rate and compared to non-exercised controls. To test cognition, volitional exercised and non-exercised controls were subjected to a repeated maze test. While both groups solved the maze more quickly over time, indicating a learning process, no differences in learning ability were observed between groups. Brain plasticity was assessed by mapping the telencephalon transcriptome, and 1,772 transcripts were differentially expressed between the volitional exercise and control group. Gene ontology (GO) analysis identified 195 and 272 GO categories with a significant overrepresentation of up- or downregulated transcripts, respectively. A multitude of these GO categories were associated with neuronal excitability, neuronal signalling, cell proliferation and neurite outgrowth (i.e. cognition-related neuronal markers). In conclusion, sustained volitional exercise promoted the expression of neuroplasticity- and neurogenesis-related genes in the telencephalon, but was not associated with significant effects on fish performance in a spatial learning task. Notably, this is the first time that swimming exercise has been directly linked to increased telencephalic neural plasticity in a teleost, and our results pave the way for future studies on exercise-induced neuroplasticity in fish.

Keywords: brain plasticity, swimming exercise, neurogenesis, Atlantic salmon, cognition, memory and learning

Introduction

Accumulating evidence shows that physical exercise can have positive effects on cognition and neural plasticity in mammals (reviewed by Cotman and Berchtold, 2002; van Praag, 2008). Specifically, both forced and voluntary running exercise have been shown to improve the cognitive performance of rodents in spatial tasks (Fordyce and Farrar, 1991; Fordyce and Wehner, 1993; van Praag et al., 1999a; Vaynman et al., 2004; van der Borght et al., 2007; Uysal et al., 2015). This effect is strongly associated with increased neurogenesis and synaptic plasticity in the mammalian hippocampus, particularly in the dentate gyrus, mediated by an increased abundance of growth factors, neurotransmitters and neurotrophic factors (reviewed by van Praag, 2008; Vivar et al., 2013; Ma et al., 2017).

Even though the link between exercise, neural plasticity and cognition is well-described in mammals, this phenomenon has not received much attention in other vertebrates, such as fish. Even though the mammalian and teleostean ancestral lines diverged around 400 million years ago, mammals and fish share many conserved neurochemical, topological and functional neural characteristics (Winberg and Nilsson, 1993; O'Connell and Hofmann, 2011; Broglio et al., 2015). Furthermore, while neurogenesis in the adult mammalian brain occurs at relatively low rates and only in discrete brain areas, fish display neurogenesis in a multitude of brain areas throughout their entire lives (Zupanc, 2006; Barker et al., 2011; Zupanc and Sîrbulescu, 2011). Therefore, it has been hypothesised that exercise-induced stimulation of neurogenesis and cognition should also occur in fish (Huntingford and Kadri, 2013), although to date, this hypothesis has not been thoroughly tested. Notably, a pilot study conducted by Luchiari and Chacon (2013) demonstrated that exhaustive swimming exercise in zebrafish (*Danio rerio*) improved their learning performance in a conditioning test within several days. Furthermore, a ten-day swim training regime promoted the expression of the cell proliferation and neurogenesis markers *basic helix-loop-helix family, member e40* (*bhlhe40*) and *growth arrest and DNA-damage inducible, beta α* (*gadd45βα*) in the brain of zebrafish larvae (Fiaz et al., 2014).

In mammals, higher functions such as cognition, perception and spatial reasoning are mostly under control of the neocortex (Jerison, 1973). In contrast to mammals, fish lack a six-layered pallium, but nonetheless, they are able to display a number of higher cognitive functions, which are under telencephalic control (Grosenick et al., 2007; Ito and Yamamoto, 2009; Bshary and Brown, 2014). Importantly, the fish telencephalon contains neural populations and networks associated with emotional and relational memory, learning and stress-reactivity, thus driving processes which show functional resemblances to processes which are under control of the limbic system in mammals (Broglio et al., 2005; Rodríguez et al., 2007; Vargas et al., 2009; O'Connell and Hofmann, 2011). Therefore, we hypothesise that exercised fish will show enhanced telencephalic neural plasticity, and that this will be associated with improved cognitive function, compared to unexercised control individuals.

Exercise-enhanced cognition in fish may have important applications in stocking, which is a conservation strategy whereby hatchery-reared fish (generally salmonids) are released into the wild to supplement local depleted populations (Maynard et al., 1995). However, the hatchery environment is impoverished compared to wild conditions, and thus, hatchery-reared fish show deviating neurobiology (Mes et al., 2018) and are behaviourally naïve (Olla et al., 1998; Huntingford, 2004) compared to wild fish, which results in low post-release survival rates of stocked fish (Johnson et al., 1996; Thorstad et al., 2011). In order to improve salmonid stocking success, hatchery managers are searching for innovative ways to improve the fitness of hatchery-reared fish through enhanced neural and cognitive development, and swimming exercise may be a suitable practice to achieve this. Therefore, the aim of the current study was to assess whether sustained swimming exercise can promote forebrain neuroplasticity and cognitive performance in Atlantic salmon (*Salmo salar*, L.) parr (juvenile fish that reside in freshwater), in order to evaluate whether swimming exercise has the potential to improve cognition in hatchery-reared salmon in stocking programs. Fish were subjected to an eight-week sustained swimming exercise regime at either a forced or volitional (where fish can choose their swimming speed) swimming speed. After training, RNA sequencing (RNA-seq) was used to map the transcriptome of the telencephalon in order to quantify the expression of neuroplasticity and neurogenesis markers. In addition, cognitive performance was assessed for the volitional swimmers and unexercised controls after three and eight weeks of swimming by means of a repeated maze test. We here report for the first time that swimming exercise leads to upregulation of key neuroplasticity-related genes in the Atlantic salmon telencephalon, and we highlight several resemblances with mammalian exercise-induced neural plasticity.

Materials and Methods

Ethics statement

This experiment was performed in accordance with Dutch law for experimentation and procedures on live animals. The experimental protocol was approved by the Animal Experimental Committee (DEC) of Wageningen University & Research (case number 2016.D-0039).

Experimental fish

Experimental fish were hatchery-reared Atlantic salmon parr, which were first generation offspring from wild-caught parents from the river Imsa, in south-western Norway. Eggs hatched in late January 2017 and fish were reared under standard hatchery conditions at the Norwegian Institute for Nature research (NINA) Research Station at Ims, Norway, in water from the adjacent river Imsa. On August 31st 2017, 450 fish were transported from the Ims hatchery to the aquaculture research facilities at Wageningen University & Research (WUR), the Netherlands. Fish were transported in groups of 45 individuals in double-layered plastic bags, containing 10 L of system water (10˚C) and 40 L of 100%

oxygen. Fish were lightly anaesthetised in Aqui-S (Scan Aqua, Årnes, Norway; 3.3 mg L^{-1}) prior to packing and during transport, which has been shown to reduce stress (Iversen and Eliassen, 2009). The bags were placed in expanded polystyrene-lined cardboard boxes and two frozen cooling elements were added, in order to keep water temperature and fish respiration rates low. Fish were in transit for a total time of 12 hours by air and road travel and no transport-related mortality was observed. After 18 days of acclimatisation at the WUR experimental facilities, fish were tagged intraperitoneally with passive integrated transponders (PIT) tags (Trovan ID100A/1.4 mini transponders) and the animals were then left to recover for an additional week before the experiment started. Fish were nine months old at the start of the experiment and eleven months old at time of final sampling. An overview of experimental procedures is provided in **Fig. 1**.

Swimming exercise regime

Fish were exercised for eight weeks at either a forced or a volitional swimming speed. At the start of the exercise regime, fish measured 123 ± 5 mm (fork length; FL) and weighed 20.8 ± 3.6 grams (mean ± SD).

In the forced exercise regime, parr were housed in a 3,600-L Brett-type swimming flume (Brett, 1964) as described by Palstra et al. (2015). In short, the flume contained two adjacent rectangular 525-L holding compartments, of which the outer compartment received a water flow at a predetermined speed, while the inner compartment did not receive a water flow and housed the control (non-exercised) fish (**Fig. 1**). During swimming, the top of the flume was sealed with a lid to minimise disturbance and light was provided in the upstream part of the compartments, with a light intensity of approximately 50 lux at the water surface. Fish in the forced swimming treatment were exercised at a continuous flow rate of 43 cm s^{-1} (3.5 body lengths (BL) s^{-1}), while flow in the control compartment was < 5 cm s^{-1} (< 0.4 BL s^{-1}). A swimming speed of 43 cm s^{-1} falls within the range of flow rates (0-65 cm s^{-1}) which are preferred by wild Atlantic salmon parr in natural streams (reviewed by Armstrong et al., 2003). Additionally, a relative flow rate of 3.5 BL s^{-1} is close to the optimal metabolic swimming speed of 3.8 BL s^{-1} for 200-mm Atlantic salmon (A.P. Palstra, unpublished data) and is approximately at 60% of the maximum swimming speed of 5.5 – 5.8 BL s^{-1} reported for size-matched hatchery-reared Atlantic salmon of this particular Imsa population (Hammenstig et al., 2014). Exercised fish distributed themselves across the bottom of the compartment and displayed rheotactic (i.e. facing the current) swimming at a constant speed.

The experimental setup for the volitional exercise regime consisted of two standard cylindrical 800-L holding tanks, of which the exercise treatment tank received a high water flow adjacent to the tank wall (**Fig. 1**). At the bottom of the exercise tank, the flow rate varied from 5 cm^{-1} (0.4 BL s^{-1}) in the centre to 27 cm^{-1} (2.2 BL s^{-1}) at the outer wall, and flow rates at the water surface were 10 cm s^{-1} (0.8 BL s^{-1}) in the centre and 36 cm s^{-1} (2.9 BL s^{-1}) at the outer wall. Thus, by positioning themselves in the tank,

fish could 'choose' their preferred swimming speed. Water flow in the tank of the controls was < 5 cm s^{-1} throughout the tank. The selected flow rates were the maximum speeds that could be achieved in the standard hatchery tanks and were well within the aerobic scope of salmon (Hammenstig et al., 2014), as well as within the preferred range of flow rates of Atlantic salmon in natural habitats (Armstrong et al., 2003). Both tanks were covered with mesh and half of the tank was covered with black foil to provide shelter. Light intensity at the water surface was approximately 45 lux. Exercised fish showed no sign of fatigue and generally displayed rheotactic swimming while holding their position, while occasionally drifting down with the current.

For all fish groups, the light cycle was maintained at 12:12 L:D throughout the experiment. Water temperature was maintained at 14.9 ± 0.45 ˚C and nutrient levels were 0.06 ± 0.05 mg NH_4 L^{-1}, 0.08 ± 0.04 mg NO_2 L^{-1} and 67.6 ± 24 mg NO_3 L^{-1} (mean ± SD). The two flume compartments which housed the forced exercise treatment and their controls housed 65 fish each, while the two cylindrical tanks which housed the volitional exercised treatment and their respective controls contained 110 fish each, yielding the same density of 2.6 kg m^{-3} for all groups. Fish were fed commercial pellets (Nutra Parr, Skretting, Stavanger, Norway) by hand, twice per day until satiation and water flow was stopped during feeding to provide equal feeding opportunities for both exercised and sedentary fish. All fish were measured and weighed after the swimming treatment and specific growth rates were calculated as follows:

$$SGR = \left(ln(BM_f) - ln(BM_i)\right) \times \frac{100}{t}$$

Where BM_f is the final body mass in g at the end of the exercise period, BM_i is the initial body mass in g at the start of the experiment and t is the experimental time in days.

Maze test

To evaluate whether swimming exercise had an effect on cognition, volitional exercised fish and their respective controls were subjected to a four-armed maze test after both three and eight weeks of swimming. We did not have the capacity to test all four groups, and as most mammalian studies report increased cognitive performance in animals subjected to voluntary exercise (e.g. van Praag et al., 1999a; van der Borght et al., 2007), we decided to test the volitional group and their controls in this study. The maze setup and procedures were modelled after methodology described by Salvanes et al. (2013). In short, 15 fish from the voluntary exercise and respective control group were randomly selected and tested in two separate mazes. The two identical mazes were partly submersed in 800-L rectangular tanks and consisted of a 38 x 38 cm arena, with a start box on one side and four transparent arms on the opposite side (**Fig. 1**). Next to the arms, an aquarium containing three salmon parr served as a social stimulus for the fish to exit the maze. Prior to the test, fish were acclimatised to the maze for 48 hours by placing all 15 fish simultaneously in the centre of the arena, while access to the start box was blocked.

During acclimatisation, all four arms were open so that the animals could exit and explore the maze and the surrounding holding tank. After acclimatisation, fish were transferred to cylindrical holding tanks (diameter 65 cm, water depth 65 cm; separate tanks for exercised and non-exercised fish) where exercised fish were subjected to a flow of approximately 17-21 cm s^{-1} while control fish received no measurable flow. The flow rate in these holding tanks was slightly lower than that in the original treatment tanks because the diameter of the maze holding tanks was smaller than that of the treatment holding tanks, and swimming in a curved path incurs additional energetic expenditure (Domenici et al., 2000). Three randomly assigned arms of the maze were then closed, leaving only one arm open, from which fish could exit the maze. During the following seven days, all fish were tested daily in the maze. For each test, an individual fish was collected from the holding tank by dip net and subsequently PIT-scanned and placed in the start box of the maze. After three minutes, the hatch to the arena was remotely opened and fish were given 5 min to enter the maze. If the fish didn't enter the maze within 5 min, it was guided into the maze using transparent plastic paddles. Once within the arena, the fish was given 5 min to find the open arm exit, and if the animal did not succeed within this time, individuals were guided towards the exit. All tests were conducted by one observer, who could not be seen by the fish while he could observe the animals through a camera that was mounted approx. 1.5 m above the maze. The number of errors and time to exit the maze were scored by the observer. This test was conducted twice, after three and eight weeks of exercise, using different individuals. The exercised and control fish were tested in the opposite maze at three and eight weeks, to rule out maze-effects.

RNA isolation and sequencing

After eight weeks of swimming, five animals per group (n = 20 total) were randomly collected and quickly anaesthetised in 2-phenoxyethanol (VWR #26244.290, 1.3 ml L^{-1}). Opercular movement ceased completely within 30 seconds, after which weight and length were recorded. Immediately after, fish were decapitated and within two minutes, the telencephalon was dissected out and placed overnight in RNAlater (Invitrogen AM7024) at 4˚C. The following day, surplus RNAlater was removed and samples were stored at -80˚C. The telencephalon tissue was then homogenised using a TissueRuptor (Qiagen, Venlo, The Netherlands) and total RNA was extracted using the miRNeasy mini kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions. Integrity and concentration of the RNA were checked on a Bioanalyzer 2100 total RNA Nano series II chip (Agilent, Amstelveen, The Netherlands) and the median RIN value was 9.0. Illumina RNA-seq libraries were prepared from 0.5 µg total RNA using the Illumina TruSeq® Stranded mRNA Library Prep kit according to the manufacturer's instructions (Illumina, San Diego, USA). All RNA-seq libraries (150-750 bp inserts) were sequenced on an Illumina HiSeq2500 sequencer as 1× 50 nucleotides single-end reads according to the manufacturer's protocol. Image analysis and base calling were done using the Illumina pipeline.

Transcript quantification

The reads were aligned to the latest version of the Atlantic salmon genome reference (ICSASG version 2, NCBI RefSeq GCF_000233375.1; Lien et al., 2016) using TopHat version 2.0.13 (Kim et al., 2013) at 'very-sensitive' default settings. Samtools version 1.2 (Li et al., 2009) was used to remove secondary alignments, i.e. alignments that meet TopHat's reporting criteria but are less likely to be correct than simultaneously reported primary alignments. Alignments to annotated exons were counted and summarised at the gene level using HTSeq-count version 0.10.0 (Anders et al., 2015) using the 'intersection-nonempty' setting.

Differential expression analysis

Raw read counts for 48,436 protein-coding genes were analysed in R version 3.4.4 (R Development Core Team, 2016) using the edgeR package version 3.20.9 (Robinson et al., 2010). Initially, read counts were normalised using the TMM method and a multidimensional scaling (MDS) plot was generated to identify outliers. After outlier removal, the read counts were normalised again, and differential expression between the four treatment groups was calculated using edgeR's recommended quasi-likelihood F-test for generalised linear models. Multidimensional scaling plots and differential expression were only calculated for genes with at least 10 aligning reads in each sample. For downstream analyses, reads per kilobase million (RPKM) expression values (normalised between samples and corrected for transcript length) were exported from edgeR. Transcripts with a false discovery rate (FDR) < 0.01 were considered to be significantly differentially expressed between treatments. The TM4 MultiExperiment Viewer version 4.9.0 (www.tm4.org) was used to visualise expression profiles in heat maps.

Gene Ontology (GO) analysis

Gene Ontology annotations for the ICSASG_v2 assembly were retrieved using the Ssa.RefSeq.db R package version 1.2 (https://rdrr.io/github/FabianGrammes/Ssa.RefSeq.db/), and overrepresentation of 'Biological Process' categories was assessed using the R package GOseq (Young et al., 2010), using the 'Wallenius' method and including correction for transcript length.

Statistical analyses

Normality and homogeneity of variance of morphometric data were assessed by Shapiro-Wilk and Levene's tests, respectively. Body mass and fork length were compared using Wilcoxon rank-sum tests, while SGR_w was compared using two-tailed Student's t-tests. Only the following useful pairwise comparisons were made: forced control *vs.* forced exercised and volitional control vs. volitional exercised. All morphometric data are presented as mean ± SEM

Maze performance was analysed according to the methodology outlined by Salvanes et al. (2013). In short, we calculated the cumulative number of errors ('Cumul.Errors') and cumulative time to find the exit of the maze ('Cumul.Exit') over successive trials for individual fish. To test for differences in the

cumulative number of errors made, linear mixed-effects models assuming first-order autocorrelation were used, with fish ID ('ID') as random effect factor to account for repeated observations of individual fish. 'Treat' (treatment: control and exercised) and 'Day' (experimental day) were specified as fixed effects. The "Anova" command within the "car" library was used to extract the main results from the model and the "lsmeans" command within the "lsmeans" package was used as a post-hoc test to compare the number of errors per trial between exercised and control fish. To test for differences in time to leave the maze, a polynomial model was employed, as described by Salvanes et al. (2013). Statistical analyses were done using R version 3.4.4 (R Development Core Team, 2016).

Results

Growth

There were no significant morphometric differences between experimental groups at the start of the experiment, with the exception that fish in the forced exercise group had a significantly higher fork length than individuals in the forced control group (W_s = 3643, Z = -2.00, p = 0.045), although there was no significant difference in body mass between these two groups (W_s = 3678, Z = -1.84, p = 0.066). After eight weeks of swimming, exercised fish had gained 27% (volitional) and 31% (forced) more body mass compared to control fish and the final body mass of exercised fish was significantly higher than that of control fish for both the volitional (32.1 ± 0.5 *vs*. 29.3 ± 0.6; W_s = 4653, Z = -3.33, p = 0.0009) and forced treatment (32.7 ± 0.7 *vs*. 29.0 ± 0.8; W_s = 1988, Z = -3.47, p = 0.0005; **Fig. 2**). Specific growth rates of exercised animals were significantly higher for both the volitional (0.73 ± 0.02 *vs*. 0.65 ± 0.02; t = 2.98, df = 149, p= 0.0033) and forced treatment groups (0.76 ± 0.02 *vs*. 0.61 ± 0.02; t = 4.58, df = 99, p < 0.0001), compared to their respective controls. Feed conversion ratios (FCR) were 2.03 and 1.95 for forced control and forced exercised, and 1.28 and 1.34 for volitional control and volitional exercised groups, respectively.

Maze test

After three weeks of treatment, there was no difference between exercised and control fish in time to exit the maze (no significant interaction effects between time and treatment (χ = 1.85, df = 2, p = 0.94) and no effect of treatment (χ = 0.01, df = 1, p = 0.92), **Fig. 3A**). Regarding the number of errors made, at day 1, there was no difference between treatments (t = -0.46, df = 28, p = 0.65) but over time, volitional swimmers made significantly fewer mistakes than control fish (interaction effect, χ = 22.4, df = 1, p < 0.001).

After eight weeks of treatment, there was again no difference between treatments in time to exit the maze (no significant interaction effects between time and treatment (χ = 0.10, df = 2, p = 0.95) and no effect of treatment (χ = 0.0003, df = 1, p = 0.99), **Fig. 3B**). Regarding the number of errors made, at day

1, there was no difference between treatments (t = -0.31, df = 27, p = 0.76) but over time, volitional swimmers made significantly more mistakes than control fish (interaction effect, χ = 19.0, df = 1, p < 0.001).

RNA-seq

In order to determine whether exercise affects brain plasticity at the cellular level, we measured gene expression levels in the telencephalon of five fish per group using Illumina RNA-seq. We obtained between 10.1 and 39.1 million reads per sample (median 17 million), of which 90.6–95.8% (median 95.4%) aligned to the salmon genome. Of all aligning reads, 63.2–79.1% (median 76.9%) could be attributed to a protein-coding gene.

As an initial quality control, we examined a MDS plot to identify possible outliers. Four samples did not cluster with their respective experimental group (see **Fig. S1**). Upon inspection, these samples were characterised by high expression of eye-related genes, suggesting a contamination with optical nerve tissue during dissection. We therefore decided to exclude these samples from all subsequent analyses, leaving three (forced exercised), four (volitional exercised and volitional control) or five (forced control) samples per treatment.

A MDS plot of the remaining samples (**Fig. 4**) shows a clear clustering of each experimental group, indicating robust gene expression changes correlated with the treatments. We therefore analysed differential expression of 27,171 genes (which does not include genes below a very low expression threshold of 10 reads per sample) between volitional swimmers and their respective controls (**Fig. 5A**) and between forced swimmers and their respective controls (**Fig. 5B**). The contrast between volitional and control groups yielded 1,772 genes differentially expressed using a false discovery rate (FDR) cutoff of 1%, of which 923 had significantly higher expression in swimmers (**Table S1)**, and 849 had significantly higher expression in control fish (**Table S2**). A selection of these genes is presented in **Table 1**.

By contrast, we found no significant differential expression of genes in the forced swimming group (**Fig. 5B**). However, when we examined the direction and magnitude of changes in gene expression for both contrasts, we observed a clear correlation trend between the changes (**Fig. 6**), indicating a similar effect on global gene expression changes in forced and volitional swimmers (Pearson correlation coefficient 0.60). In other words, for the vast majority of genes significantly overexpressed in volitional swimmers (red dots on the upper right in **Fig. 6**), there was a non-significant overexpression in forced swimmers compared to forced controls. While similar expression profiles are thus found between forced and volitional swimmers, the magnitude of the gene expression changes does appear to be larger in the volitional swimmers group (**Fig. 6**).

Functional overrepresentation

To summarise which biological processes are over- or underexpressed in the volitional swimmers group, we performed a Gene Ontology (GO) category overrepresentation test on the sets of significantly differentially expressed genes between volitional exercised fish and their controls. In total, 194 (**Table S3**) and 271 (**Table S4**) GO categories showed a significant overrepresentation of upregulated and downregulated genes, respectively ($p < 0.05$). The GO categories which related to neuroplasticity, neurogenesis or behavioural pathways involved with cognition were selected and relevant GO categories with a significant overrepresentation of upregulated genes are presented in **Table 2**, while downregulated GO categories are presented in **Table 3**. The heat map in **Fig. 7** provides a visual overview of the expression of selected neuroplasticity-related genes in all samples, showing a clustering of expression by treatment.

Discussion

We here report, for the first time in a fish species, that sustained swimming exercise at a volitional swimming speed increased the expression of neuroplasticity- and cell proliferation-related genes in the telencephalon transcriptome of juvenile Atlantic salmon. However, we did not find indications that increased neuroplasticity in exercised individuals led to enhanced cognitive performance in a spatial orientation task.

Exercised fish in both the volitional and forced regimes showed significantly enhanced growth rates compared to unexercised controls. Exercise-induced growth is a well-described phenomenon across several orders of teleosts, particularly in salmonids, provided that the exercise speed is close to the optimal metabolic swimming speed (U_{opt}), i.e. the swimming speed at which a fish spends the least amount of energy per unit distance travelled (reviewed by Davison and Herbert, 2013). At swimming speeds below U_{opt}, fish may divert excess energy away from growth and towards agonistic behaviour (reviewed by Huntingford and Kadri, 2013), while speeds above U_{opt} may induce stress and chronic fatigue, with negative consequences for growth (Davison and Herbert, 2013). Thus, supra- and suboptimal swimming speeds compromise growth in salmonids, and the occurrence of exercise-enhanced growth in the current experiment, both under volitional and forced exercise regimes, suggests that our experimental treatment had beneficial effects on the physiology of the fish and did not lead to chronic stress.

After eight weeks of swimming, 1,772 transcripts in the telencephalon were differentially expressed in fish exercised at a volitional swimming speed, compared to unexercised controls. Gene ontology analysis attributed these differences in transcript abundance to processes relating to neural plasticity, such as dendritic spine development and synaptic plasticity, as well as processes related to cell proliferation and apoptosis. In mammals, both voluntary and forced exercise training regimes have been shown to stimulate hippocampal abundance of neurotrophins such as brain-derived neurotrophic factor

(*BDNF*), as well as memory and learning processes such as long-term potentiation (LTP; Bliss and Collingridge, 1993; O'Callaghan et al., 2007; Uysal et al., 2015). Thus, we had expected to also find significant stimulatory effects of swimming on neural plasticity in fish exercised at a forced speed. However, in the forced swimming treatments, no transcripts were significantly differentially expressed between exercised and control animals. It is plausible that the lack of any significant differences in gene expression between forced exercised and control fish is a consequence of a smaller amplitude of the effects (**Fig. 6**). In addition, the statistical power in this contrast may have been reduced by the (asymmetrical) removal of two outlier samples from the forced swimmers group. Interestingly, gene expression patterns between forced and volitional swimmers were highly correlated: for the vast majority of genes significantly overexpressed in volitional swimmers, there was a non-significant overexpression in forced swimmers compared to forced controls. This is suggestive of a similar biological effect in both volitional and forced exercised groups, but with more pronounced effects in the volitional exercise treatment. Mammalian studies have demonstrated that forcing animals to exercise at too high intensity can cause stress and adverse effects on neural plasticity, evidenced by the depressed induction of hippocampal *BDNF* mRNA expression (Soya et al., 2007). However, it seems unlikely that fish in the forced exercise treatment in the current experiment were exercised at too high intensity and that this may explain the observed smaller effects on neural plasticity, as forced swimmers displayed substantial exercise-enhanced growth, similar to fish in the volitional treatment.

Telencephalic gene expression profiles in the volitional exercised fish showed several similarities with reported upregulated hippocampal genes in exercised mammals. Mammalian studies are starting to uncover the molecular pathways underlying exercise-induced neurogenesis and synaptic plasticity (Molteni et al., 2002; Lista and Sorrentino, 2010). In summary, physical activity in mammals first leads to an increased abundance of neurotrophins, such as BDNF and insulin-like growth factor (IGF; reviewed by Vivar et al., 2013). Subsequently, BDNF can directly promote neurogenesis, or it may activate signal transduction pathways through signalling molecules such as calcium/calmodulin-dependent protein kinase II (CAMK-II), mitogen-activated protein kinase (MAPK), protein kinase C (PKC) and cAMP response element binding (CREB) protein (Molteni et al., 2002; Farmer et al., 2004), which in turn stimulate neural processes such as synaptogenesis and LTP (reviewed by Lista and Sorrentino, 2010). Furthermore, synaptogenesis is enhanced by synaptic trafficking molecules such as synaptotagmin and syntaxin, which are promoted through CAMK-II after activation by BDNF or IGF (Molteni et al., 2002). In exercised fish in the current study, we observed an upregulation of several genes within these pathways, such as synaptotagmin, syntaxin CAMK-II, MAPK, PKC and CREB, as well as two IGF receptor-related transcripts. Molteni et al. (2002) further report that running exercise activates the mammalian hippocampal glutamatergic system and suppresses the gamma-aminobutyric acid (GABA)ergic system. Similarly, exercised fish in our experiment showed increased expression of several glutamate receptor transcripts and reduced expression of several GABA receptor transcripts,

although effects on the GABAergic system are somewhat ambivalent, as we observed concurrent upregulation of several GABA-receptor subunit transcripts in exercised individuals. In summary, there are several parallels between the teleostean and mammalian neural response to exercise in processes regarding synaptic trafficking, signal transduction and the glutamatergic and GABAergic systems. These findings suggest that the molecular pathways which underlie exercise-induced neuroplasticity are at least partly conserved between mammals and teleost fish.

The neurotrophin BDNF is a well-characterised neural growth factor which is important for synaptic plasticity and neural survival (Mattson et al., 2004; Shors et al., 2012; Gray et al., 2013; Vivar et al., 2013). In exercised mammals, *BDNF* shows a robust upregulation in the dentate gyrus of the hippocampus (Molteni et al., 2002; Vaynman et al., 2004), as well as in other brain regions such as the amygdala (Liu et al., 2009). Furthermore, BDNF plays a key role in activating the signal transduction pathways which drive increased neural plasticity (Molteni et al., 2002; Lista and Sorrentino, 2010). Surprisingly, while we observed increased expression of many of these neuroplasticity-related signalling molecules in exercised fish, we did not observe an increased abundance of *bdnf* itself. Importantly, we only assessed the telencephalon transcriptome after eight weeks of swimming exercise, thus mapping the chronic effects of swimming. It is possible that telencephalic *bdnf* abundance increases immediately following the onset of swimming and subsequently decreases to basal levels again, as has been observed in salmon following transfer to a holding tank enriched with physical structures (L.O.E. Ebbesson, pers. comm.). To verify this possibility, future studies should perform a time series and quantify *bdnf* transcript levels at several hours, days and weeks after the onset of exercise. Furthermore, mammalian studies predominantly observe increased *bdnf*-abundance specifically in the hippocampus of exercised animals, while here, we mapped the transcriptome of the entire telencephalon. The fish telencephalon is comprised of a large number of neural subregions, each with specific functionalities (Northcutt, 2006; Vargas et al., 2009), and expression profiles of neuroplasticity markers can by highly region-specific (Mes et al., 2018). Notably, the dorsolateral (Dl) part of the fish telencephalon has been identified as functional equivalent to the mammalian hippocampus (Portavella et al., 2004; Vargas et al., 2009) and plays an important role in spatial memory in fish (Rodríguez et al., 2007; Broglio et al., 2010; Broglio et al., 2015). It is thus possible that region-specific trends, such as increased *bdnf* expression, might occur in specific neural subregions, such as the Dl, but that these trends are not registered in the whole-tissue sample used for RNA-seq in this study. Future studies should therefore consider specific mapping of neuroplasticity markers in distinct telencephalic subregions such as the Dl, and prioritise the quantification of established markers for running-induced neuroplasticity in mammals, such as *bdnf*.

The transcriptome of voluntary exercised animals revealed a significant overrepresentation of downregulated genes in several GO categories related to apoptosis. This is an interesting observation, as mammalian work has uncovered that, aside from promoting neurogenesis, exercise also increases

cell survival (van Praag et al., 1999b; van Praag et al., 2007) and can inhibit neuronal apoptosis, particularly in aging animals (Kim et al., 2010b) or individuals with traumatic brain injury (Kim et al., 2010a; Itoh et al., 2011). Our observation that swimming exercise may also affect neuronal apoptosis in fish requires further investigation, both in healthy animals and individuals with neural damage, as fish with neural damage show an incredible capacity for neural regeneration in the central nervous system (Kaslin et al., 2008).

In rodents, positive effects of exercise on spatial learning have been reported for both forced and voluntary exercise treatments (e.g. Fordyce and Farrar, 1991; Fordyce and Wehner, 1993; van Praag et al., 1999a; van der Borght et al., 2007). In our experiment however, despite the observed increased telencephalic expression of neuroplasticity markers in fish exercised at a volitional swimming speed, we did not find enhanced spatial memory performance of exercised individuals in the maze test. A lack of improved spatial memory performance in exercised fish apparently contradicts our finding that exercised animals show increased telencephalic neural plasticity, but as stated before, teleostean spatial memory is under control of the dorsolateral pallium (Rodríguez et al., 2002; Broglio et al., 2005), and we cannot ascertain whether the increased expression of neural plasticity markers in the entire telencephalon is also manifested in the Dl. Another factor to consider is that while in mammalian models, such as rodents, spatial memory tasks such as the Morris water maze (Morris, 1984) are well-defined, standardised and widely used (Vorhees and Williams, 2014), this is not the case for most behavioural tests used in fish research. For example, the current maze protocol, replicated from methodology described by Salvanes et al. (2013), involves considerable handling and air exposure of the fish prior to testing, which inadvertently causes stress and may negatively affect the cognitive performance of the animals. Furthermore, because we are interested in potential applications of exercise-enhanced cognition in stocking programs, we chose to use first-generation offspring from wild salmon in the current experiment. A possible negative consequence of working with non-domesticated animals is that handling stress may have a stronger negative affect on their behaviour, compared to a more domesticated fish species. Therefore, we suggest that future studies consider subjecting a model fish species, such as zebrafish, to an established spatial memory task such as the T-maze (Kalueff et al., 2014) or three-chamber task in order to further elucidate the possible stimulatory effects of exercise on cognition in fish (Levin, 2011).

A better understanding of the effects of swimming exercise on neuroplasticity in a model fish species may have further ramifications for human disease research, as an important application of mammalian exercise-induced neuroplasticity is its potential to prevent cognitive decline, particularly in the context of aging and neurodegenerative diseases (reviewed by Ma et al., 2017). The use of small model fish such as zebrafish has a number of advantages over the traditional rodent models, such as low housing costs and a short reproductive cycle (Lieschke and Currie, 2007; Kalueff et al., 2014), and as a result the zebrafish is becoming an increasingly popular model to study neurodegenerative diseases (Santana

et al., 2012; Newman et al., 2014). Our findings, combined with the observation of Fiaz et al. (2014) that swim training can increase the expression of cell proliferation and neurogenesis markers in the zebrafish larval brain, further supports the concept that zebrafish may be a promising new model for exercise-induced neuroplasticity research.

Our finding that sustained swimming exercise promotes neural plasticity in Atlantic salmon may have substantial implications for fish stocking practices. Interestingly, a number of studies have previously investigated the effects of swimming exercise on post-release survival of stocked salmonids (e.g. Burrows, 1969; Cresswell and Williams, 1983; Evenson and Ewing, 1993; Hoffnagle et al., 2006), with most of these studies reporting positive effects, provided that fish were exercised at a moderate to high swimming intensity (reviewed by Maynard et al., 1995). While these studies have mainly focused on the role of increased cardiovascular performance and exercise-enhanced growth on post-release survival, our results give a first insight into the potential neural mechanisms behind improved stocking success in exercised salmonids. Thus, the application of exercise regimes in hatchery rearing conditions should be considered as a potential methodology to improve salmonid fitness in stocking programs.

In conclusion, we report that eight weeks of swimming exercise at a volitional swimming speed increased the expression of telencephalic neuroplasticity and cell proliferation genes in Atlantic salmon. We are among the first to study the effects of exercise on neuroplasticity in fish and our results uncover several parallels with mammalian studies, such as exercise-induced activation of the glutamatergic system, upregulation of signal transduction (e.g. CAMK, MAPK, PKC) and synaptic trafficking markers (e.g. synaptotagmin), and the downregulation of apoptosis. Future studies should perform additional cognitive tests and target specific neural plasticity markers in distinct telencephalic neural subpopulations in exercised individuals, to shed more light on the mechanisms behind exercise-induced neuroplasticity in fish, and to explore its potential application to animal welfare, and as a model for understanding human neural disease mechanisms.

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References

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* 31(2)**,** 166-169. doi: 10.1093/bioinformatics/btu638.

Armstrong, J.D., Kemp, P.S., Kennedy, G.J.A., Ladle, M., and Milner, N.J. (2003). Habitat requirements of Atlantic salmon and brown trout in rivers and streams. *Fish. Res.* 62(2)**,** 143-170. doi: 10.1016/s0165-7836(02)00160-1.

Barker, J., M., Boonstra, R., and Wojtowicz, J.M. (2011). From pattern to purpose: How comparative studies contribute to understanding the function of adult neurogenesis. *Eur. J. Neurosci.* 34(6)**,** 963-977. doi: 10.1111/j.1460-9568.2011.07823.x.

Bliss, T.V.P., and Collingridge, G.L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* 361**,** 31-39. doi: 10.1038/361031a0.

Brett, J.R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can.* 21(5)**,** 1183-1226. doi: 10.1139/f64-103.

Broglio, C., Gómez, A., Durán, E., Ocaña, F.M., Jiménez-Moya, F., Rodríguez, F., et al. (2005). Hallmarks of a common forebrain vertebrate plan: Specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. *Brain Res. Bull.* 66(4)**,** 277-281. doi: 10.1016/j.brainresbull.2005.03.021.

Broglio, C., Martín-Monzón, I., Ocaña, F., Gómez, A., Durán, E., Salas, C., et al. (2015). Hippocampal pallium and map-like memories through vertebrate evolution. *J. Behav. Brain. Sci* 5(3)**,** 109-120. doi: 10.4236/jbbs.2015.53011.

Broglio, C., Rodríguez, F., Gómez, A., Arias, J.L., and Salas, C. (2010). Selective involvement of the goldfish lateral pallium in spatial memory. *Behav. Brain Res.* 210(2)**,** 191-201. doi: 10.1016/j.bbr.2010.02.031.

Bshary, R., and Brown, C. (2014). Fish cognition. *Curr. Biol.* 24(19)**,** R947-R950. doi: 10.1016/j.cub.2014.08.043.

Burrows, R.E. (1969). The influence of fingerling quality on adult salmon survivals. *Trans. Am. Fish. Soc.* 98(4)**,** 777-784. doi: 10.1577/1548-8659(1969)98[777:TIOFQO]2.0.CO;2.

Cotman, C.W., and Berchtold, N.C. (2002). Exercise: A behavioral intervention to enhance brain health and plasticity. *Trends Neurosci.* 25(6)**,** 295-301. doi: 10.1016/S0166-2236(02)02143-4.

Cresswell, R.C., and Williams, R. (1983). Post-stocking movements and recapture of hatchery-reared trout released into flowing waters - effect of prior acclimation to flow. *J. Fish Biol.* 23(3)**,** 265-276. doi: 10.1111/j.1095-8649.1983.tb02904.x.

Davison, W., and Herbert, N.A. (2013). "Swimming-enhanced growth," in *Swimming physiology of fish: Towards using exercise to farm a fit fish in sustainable aquaculture,* eds. P.A. Palstra & V.J. Planas. (Berlin: Springer Berlin Heidelberg), 177-202.

Domenici, P., Steffensen, J.F., and Batty, R.S. (2000). The effect of progressive hypoxia on swimming activity and schooling in Atlantic herring. *J. Fish Biol.* 57(6)**,** 1526-1538. doi: 10.1111/j.1095-8649.2000.tb02229.x.

Evenson, M.D., and Ewing, R.D. (1993). Effect of exercise of juvenile winter steelhead on adult returns to Cole rivers hatchery, Oregon. *Prog. Fish Cult.* 55(3)**,** 180-183. doi: 10.1577/1548-8640(1993)055<0180:EOEOJW>2.3.CO;2.

Farmer, J., Zhao, X., van Praag, H., Wodtke, K., Gage, F.H., and Christie, B.R. (2004). Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male sprague–dawley rats *in vivo*. *Neuroscience* 124(1)**,** 71-79. doi: 10.1016/j.neuroscience.2003.09.029.

Fiaz, A.W., Léon-Kloosterziel, K.M., van Leeuwen, J.L., and Kranenbarg, S. (2014). Exploring the effect of exercise on the transcriptome of zebrafish larvae (*Danio rerio*). *J. Appl. Ichthyol.* 30(4)**,** 728-739. doi: 10.1111/jai.12509.

Fordyce, D.E., and Farrar, R.P. (1991). Enhancement of spatial learning in F344 rats by physical activity and related learning-associated alterations in hippocampal and cortical cholinergic functioning. *Behav. Brain Res.* 46(2)**,** 123-133. doi: 10.1016/S0166-4328(05)80105-6.

538 Fordyce, D.E., and Wehner, J.M. (1993). Physical activity enhances spatial learning performance with
539 an associated alteration in hippocampal protein kinase C activity in C57BL/6 and DBA/2 mice.
540 *Brain Res.* 619(1)**,** 111-119. doi: 10.1016/0006-8993(93)91602-O.
541 Gray, J.D., Milner, T.A., and McEwen, B.S. (2013). Dynamic plasticity: The role of glucocorticoids,
542 brain-derived neurotrophic factor and other trophic factors *Neuroscience* 239**,** 214-227. doi:
543 10.1016/j.neuroscience.2012.08.034.
544 Grosenick, L., Clement, T.S., and Fernald, R.D. (2007). Fish can infer social rank by observation alone.
545 *Nature* 445**,** 429-432. doi: 10.1038/nature05511.
546 Hammenstig, D., Sandblom, E., Axelsson, M., and Johnsson, J.I. (2014). Effects of rearing density and
547 dietary fat content on burst-swim performance and oxygen transport capacity in juvenile Atlantic
548 salmon *Salmo salar*. *J. Fish Biol.* 85(4)**,** 1177-1191. doi: 10.1111/jfb.12511.
549 Hoffnagle, T.L., Carmichael, R.W., and Keniry, P.J. (2006). The effect of moderately increased and
550 variable raceway flow rates on juvenile physiology, survival, and adult return of hatchery-reared
551 Chinook salmon. *Trans. Am. Fish. Soc.* 135(6)**,** 1567-1577. doi: 10.1577/T05-246.1.
552 Huntingford, F., and Kadri, S. (2013). "Exercise, Stress and Welfare," in *Swimming physiology of fish:*
553 *Towards using exercise to farm a fit fish in sustainable aquaculture,* eds. P.A. Palstra & V.J.
554 Planas. (Berlin, Heidelberg: Springer Berlin Heidelberg), 161-174.
555 Huntingford, F.A. (2004). Implication of domestication and rearing conditions for the behaviour of
556 cultivated fishes. *J. Fish Biol.* 65**,** 122-142. doi: 10.1111/j.1095-8649.2004.00562.x.
557 Ito, H., and Yamamoto, N. (2009). Non-laminar cerebral cortex in teleost fishes? *Biol. Lett.* 5(1)**,** 117-
558 121. doi: 10.1098/rsbl.2008.0397.
559 Itoh, T., Imano, M., Nishida, S., Tsubaki, M., Hashimoto, S., Ito, A., et al. (2011). Exercise inhibits
560 neuronal apoptosis and improves cerebral function following rat traumatic brain injury. *J. Neural*
561 *Transm.* 118(9)**,** 1263-1272. doi: 10.1007/s00702-011-0629-2.
562 Iversen, M., and Eliassen, R.A. (2009). The effect of AQUI-S® sedation on primary, secondary, and
563 tertiary stress responses during salmon smolt, *Salmo salar* L., transport and transfer to sea. *J. World*
564 *Aquacult. Soc.* 40(2)**,** 216-225. doi: 10.1111/j.1749-7345.2009.00244.x.
565 Jerison, H. (1973). *Evolution of the brain and intelligence.* Acedemic Press, New York.
566 Johnson, J.H., McKeon, J.E., and Dropkin, D.S. (1996). Comparative diets of hatchery and wild Atlantic
567 salmon smolts in the Merrimack river. *N. Am. J. Fish. Manage.* 16(2)**,** 440-444. doi: 10.1577/1548-
568 8675(1996)016<0440:cdohaw>2.3.co;2.
569 Kalueff, A.V., Stewart, A.M., and Gerlai, R. (2014). Zebrafish as an emerging model for studying
570 complex brain disorders. *Trends Pharmacol. Sci.* 35(2)**,** 63-75. doi: 10.1016/j.tips.2013.12.002.
571 Kaslin, J., Ganz, J., and Brand, M. (2008). Proliferation, neurogenesis and regeneration in the non-
572 mammalian vertebrate brain. *Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci.* 363(1489)**,** 101-122.
573 doi: 10.1098/rstb.2006.2015.
574 Kim, D.-H., Ko, I.-G., Kim, B.-K., Kim, T.-W., Kim, S.-E., Shin, M.-S., et al. (2010a). Treadmill
575 exercise inhibits traumatic brain injury-induced hippocampal apoptosis. *Physiol. Behav.* 101(5)**,**
576 660-665. doi: 10.1016/j.physbeh.2010.09.021.
577 Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2:
578 Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions.
579 *Genome Biol.* 14(4)**,** R36. doi: 10.1186/gb-2013-14-4-r36.
580 Kim, S.-E., Ko, I.-G., Kim, B.-K., Shin, M.-S., Cho, S., Kim, C.-J., et al. (2010b). Treadmill exercise
581 prevents aging-induced failure of memory through an increase in neurogenesis and suppression of
582 apoptosis in rat hippocampus. *Exp. Gerontol.* 45(5)**,** 357-365. doi: 10.1016/j.exger.2010.02.005.
583 Levin, E.D. (2011). Zebrafish assessment of cognitive improvement and anxiolysis: Filling the gap
584 between in vitro and rodent models for drug development. *Rev. Neuroscience.* 22(1)**,** 75-84. doi:
585 10.1515/RNS.2011.009.
586 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009). The Sequence
587 Alignment/Map format and SAMtools. *Bioinformatics* 25(16)**,** 2078-2079. doi:
588 10.1093/bioinformatics/btp352.
589 Lien, S., Koop, B.F., Sandve, S.R., Miller, J.R., Kent, M.P., Nome, T., et al. (2016). The Atlantic salmon
590 genome provides insights into rediploidization. *Nature* 533**,** 200-205. doi: 10.1038/nature17164.
591 Lieschke, G.J., and Currie, P.D. (2007). Animal models of human disease: zebrafish swim into view.
592 *Nat. Rev. Genet.* 8**,** 353-367. doi: 10.1038/nrg2091.

Lista, I., and Sorrentino, G. (2010). Biological mechanisms of physical activity in preventing cognitive decline. *Cell. Mol. Neurobiol.* 30(4)**,** 493-503. doi: 10.1007/s10571-009-9488-x.

Liu, Y.-F., Chen, H.-i., Wu, C.-L., Kuo, Y.-M., Yu, L., Huang, A.M., et al. (2009). Differential effects of treadmill running and wheel running on spatial or aversive learning and memory: roles of amygdalar brain-derived neurotrophic factor and synaptotagmin I. *J. Physiol.* 587(13)**,** 3221-3231. doi: 10.1113/jphysiol.2009.173088.

Luchiari, A.C., and Chacon, D.M.M. (2013). Physical exercise improves learning in zebrafish, *Danio rerio*. *Behav. Process.* 100**,** 44-47. doi: 10.1016/j.beproc.2013.07.020.

Ma, C.-L., Ma, X.-T., Wang, J.-J., Liu, H., Chen, Y.-F., and Yang, Y. (2017). Physical exercise induces hippocampal neurogenesis and prevents cognitive decline. *Behav. Brain Res.* 317**,** 332-339. doi: 10.1016/j.bbr.2016.09.067.

Mattson, M.P., Maudsley, S., and Martin, B. (2004). BDNF and 5-HT: A dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* 27(10)**,** 589-594. doi: 10.1016/j.tins.2004.08.001.

Maynard, D.J., Flagg, T.A., and Mahnken, C.V.W. (1995). "A review of seminatural culture strategies for enhancing the postrelease survival of anadromous salmonids", in: *Am. Fish. Soc. Symp.*

Mes, D., Von Krogh, K., Gorissen, M., Mayer, I., and Vindas, M.A. (2018). Neurobiology of wild and hatchery-reared Atlantic salmon: How nurture drives neuroplasticity. *Front. Behav. Neurosci.* doi: 10.3389/fnbeh.2018.00210.

Molteni, R., Ying, Z., and Gómez-Pinilla, F. (2002). Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. *Eur. J. Neurosci.* 16(6)**,** 1107-1116. doi: 10.1046/j.1460-9568.2002.02158.x.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* 11(1)**,** 47-60. doi: 10.1016/0165-0270(84)90007-4.

Newman, M., Ebrahimie, E., and Lardelli, M. (2014). Using the zebrafish model for Alzheimer's disease research. *Front. Genet.* 5**,** 189. doi: 10.3389/fgene.2014.00189.

Northcutt, R.G. (2006). Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *J. Comp. Neurol.* 494(6)**,** 903-943. doi: 10.1002/cne.20853.

O'Connell, L.A., and Hofmann, H.A. (2011). The vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *J. Comp. Neurol.* 519(18)**,** 3599-3639. doi: 10.1002/cne.22735.

O'Callaghan, R.M., Ohle, R., and Kelly, Á.M. (2007). The effects of forced exercise on hippocampal plasticity in the rat: A comparison of LTP, spatial- and non-spatial learning. *Behav. Brain Res.* 176(2)**,** 362-366. doi: 10.1016/j.bbr.2006.10.018.

Olla, B.L., Davis, M.W., and Ryer, C.H. (1998). Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bull. Mar. Sci.* 62(2)**,** 531-550.

Palstra, A.P., Mes, D., Kusters, K., Roques, J.A., Flik, G., Kloet, K., et al. (2015). Forced sustained swimming exercise at optimal speed enhances growth of juvenile yellowtail kingfish (*Seriola lalandi*). *Front. Physiol.* 5**,** 506. doi: 10.3389/fphys.2014.00506.

Portavella, M., Torres, B., and Salas, C. (2004). Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. *J. Neurosci.* 24(9)**,** 2335-2342. doi: 10.1523/jneurosci.4930-03.2004.

R Development Core Team (2016). R: A language and enrivonment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/*.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1)**,** 139-140. doi: 10.1093/bioinformatics/btp616.

Rodríguez, F., Broglio, C., Durán, E., Gómez, A., and Salas, C. (2007). "Neural mechanisms of learning in teleost fish," in *Fish Cognition and Behavior,* eds. C. Brown, K. Laland & J. Krause. Blackwell Publishing.

Rodríguez, F., López, J.C., Vargas, J.P., Gómez, Y., Broglio, C., and Salas, C. (2002). Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. *J. Neurosci.* 22(7)**,** 2894-2903. doi: 10.1523/jneurosci.22-07-02894.2002.

Salvanes, A.G., Moberg, O., Ebbesson, L.O., Nilsen, T.O., Jensen, K.H., and Braithwaite, V.A. (2013). Environmental enrichment promotes neural plasticity and cognitive ability in fish. *Proc. R. Soc. B* 280(1767)**,** 20131331. doi: 10.1098/rspb.2013.1331.

Santana, S., Rico, E.P., and Burgos, J.S. (2012). Can zebrafish be used as animal model to study Alzheimer's disease? *Am. J. Neurodegener. Dis.* 1(1)**,** 32-48.

Shors, T.J., Anderson, M.L., Curlik, D.M., and Nokia, M.S. (2012). Use it or lose it: How neurogenesis keeps the brain fit for learning. *Behav. Brain Res.* 227(2)**,** 450-458. doi: 10.1016/j.bbr.2011.04.023.

Soya, H., Nakamura, T., Deocaris, C.C., Kimpara, A., Iimura, M., Fujikawa, T., et al. (2007). BDNF induction with mild exercise in the rat hippocampus. *Biochem. Biophys. Res. Commun.* 358(4)**,** 961-967. doi: 10.1016/j.bbrc.2007.04.173.

Thorstad, E.B., Uglem, I., Arechavala-Lopez, P., Økland, F., and Finstad, B. (2011). Low survival of hatchery-released Atlantic salmon smolts during initial river and fjord migration. *Boreal Environ. Res.* 16**,** 115-120.

Uysal, N., Kiray, M., Sisman, A., Camsari, U., Gencoglu, C., Baykara, B., et al. (2015). Effects of voluntary and involuntary exercise on cognitive functions, and VEGF and BDNF levels in adolescent rats. *Biotech. Histochem.* 90(1)**,** 55-68. doi: 10.3109/10520295.2014.946968.

van der Borght, K., Havekes, R., Bos, T., Eggen, B.J., and van der Zee, E.A. (2007). Exercise improves memory acquisition and retrieval in the Y-maze task: Relationship with hippocampal neurogenesis. *Behav. Neurosci.* 121(2)**,** 324-334. doi: 10.1037/0735-7044.121.2.324.

van Praag, H. (2008). Neurogenesis and exercise: Past and future directions. *Neuromolecular Med.* 10(2)**,** 128-140. doi: 10.1007/s12017-008-8028-z.

van Praag, H., Christie, B.R., Sejnowski, T.J., and Gage, F.H. (1999a). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc. Natl. Acad. Sci.* 96(23)**,** 13427-13431. doi: 10.1073/pnas.96.23.13427.

van Praag, H., Kempermann, G., and Gage, F.H. (1999b). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* 2(3)**,** 266-270. doi: 10.1038/6368.

van Praag, H., Lucero, M.J., Yeo, G.W., Stecker, K., Heivand, N., Zhao, C., et al. (2007). Plant-derived flavanol (−)epicatechin enhances angiogenesis and retention of spatial memory in mice. *J. Neurosci.* 27(22)**,** 5869-5878. doi: 10.1523/jneurosci.0914-07.2007.

Vargas, J.P., López, J.C., and Portavella, M. (2009). What are the functions of fish brain pallium? *Brain Res. Bull.* 79(6)**,** 436-440. doi: 10.1016/j.brainresbull.2009.05.008.

Vaynman, S., Ying, Z., and Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur. J. Neurosci.* 20(10)**,** 2580-2590. doi: 10.1111/j.1460-9568.2004.03720.x.

Vivar, C., Potter, M.C., and van Praag, H. (2013). "All about running: Synaptic plasticity, growth factors and adult hippocampal neurogenesis," in *Neurogenesis and Neural Plasticity,* eds. C. Belzung & P. Wigmore. (Berlin, Heidelberg: Springer Berlin Heidelberg), 189-210.

Vorhees, C.V., and Williams, M.T. (2014). Assessing spatial learning and memory in rodents. *ILAR Journal* 55(2)**,** 310-332. doi: 10.1093/ilar/ilu013.

Winberg, S., and Nilsson, G.E. (1993). Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. *Comp. Biochem. Physiol., C: Pharmacol. Toxicol. Endocrinol.* 106(3)**,** 597-614. doi: 10.1016/0742-8413(93)90216-8.

Young, M.D., Wakefield, M.J., Smyth, G.K., and Oshlack, A. (2010). Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biol.* 11(2)**,** R14. doi: 10.1186/gb-2010-11-2-r14.

Zupanc, G.K.H. (2006). Neurogenesis and neuronal regeneration in the adult fish brain. *J. Comp. Physiol., A* 192(6)**,** 649-670. doi: 10.1007/s00359-006-0104-y.

Zupanc, G.K.H., and Sîrbulescu, R.F. (2011). Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. *Eur. J. Neurosci.* 34(6)**,** 917-929. doi: 10.1111/j.1460-9568.2011.07854.x.

Table 1. Selection of significantly (false discovery rate (FDR) < 0.01) differentially expressed genes in juvenile Atlantic salmon exercised at a volitional swimming speed, compared to unexercised controls. Expression is given as fold change (FC) difference in exercised:control individuals.

Product	Gene name	FC
Synaptic trafficking		
Synaptotagmin		
synaptotagmin-7-like	LOC106592751	3.11
synaptotagmin-11-like	LOC106569883	2.06
synaptotagmin XVII	syt17	1.97
synaptotagmin-7-like	LOC106587514	1.67
synaptotagmin-7-like	LOC106562326	1.42
Syntaxin		
syntaxin binding protein 5 (tomosyn)	stxbp5	1.71
syntaxin-12-like	LOC106578562	0.78
Signal transduction		
CAM kinases		
calcium/calmodulin-dependent protein kinase type II subunit beta-like	LOC106570801	2.23
calmodulin-binding transcription activator 2-like	LOC106565081	1.97
calcium/calmodulin-dependent protein kinase kinase 1-like	LOC106603934	1.94
calcium/calmodulin-dependent protein kinase type 1-like	LOC106583531	1.91
striatin%2C calmodulin binding protein 4	strn4	1.66
calcium/calmodulin-dependent protein kinase type II subunit beta-like	LOC106569240	1.50
calcium/calmodulin-dependent protein kinase kinase 2%2C beta	camkk2	1.37
MAP kinases		
mitogen-activated protein kinase 6-like	LOC106587736	1.67
mitogen-activated protein kinase kinase kinase 12	map3k12	1.63
mitogen-activated protein kinase kinase kinase kinase 3-like	LOC106577155	1.61
mitogen-activated protein kinase-activated protein kinase 5	mapkapk5	1.34
mitogen-activated protein kinase 6-like	LOC106594832	1.33
Protein kinase C		
protein kinase C-binding protein NELL1-like	LOC106561902	1.66
protein kinase C beta type	LOC106594520	1.66
CREB		
CREB-regulated transcription coactivator 3-like	LOC106562618	1.85
CREB-binding protein-like	LOC106589530	1.42
Glutamatergic system		
metabotropic glutamate receptor 5-like	LOC106563916	3.89
metabotropic glutamate receptor 5-like	LOC106581415	2.53
glutamate receptor 2-like	LOC106595266	2.38
glutamate receptor ionotropic, NMDA 2B-like	LOC106601156	2.05
glutamate receptor ionotropic, AMPA 4	gria4	1.52
glutamate decarboxylase 2	gad2	1.51
glutamate receptor ionotropic, kainate 5-like	LOC106576347	1.45
glutamate receptor ionotropic, delta-2-like	LOC106580171	1.28
GABAergic system		
gamma-aminobutyric acid receptor-associated protein-like 2	LOC106587940	0.68
gamma-aminobutyric acid receptor-associated protein	gbrap	0.72
gamma-aminobutyric acid receptor-associated protein-like 1	grl1	0.73
gamma-aminobutyric acid receptor-associated protein	LOC106602900	0.74
gamma-aminobutyric acid receptor-associated protein-like 1	LOC106576832	0.75
gamma-aminobutyric acid receptor-associated protein-like	LOC106577557	0.77
gamma-aminobutyric acid receptor subunit alpha-5-like	LOC106563719	0.77
gamma-aminobutyric acid receptor subunit beta-2-like	LOC106603846	2.26
gamma-aminobutyric acid type B receptor subunit 1-like	LOC106570681	2.24

gamma-aminobutyric acid receptor subunit gamma-1-like	LOC106610931	2.04
gamma-aminobutyric acid type B receptor subunit 2-like	LOC106570447	1.89
gamma-aminobutyric acid receptor subunit beta-1-like	LOC106610929	1.42
Growth factors		
IGF		
insulin-like growth factor 1 receptor	igf1r	2.38
insulin-like growth factor 1 receptor	LOC106592162	1.88
FGF		
fibroblast growth factor receptor 2	fgfr2	1.36
fibroblast growth factor receptor substrate 2-like	LOC106561545	0.68

699

Table 2. Selection of gene ontology (GO) categories with a significant ($p < 0.05$) overrepresentation of upregulated genes in fish exercised at a volitional swimming speed, sorted by theme, i.e. np: neuroplasticity, cp: cell proliferation, beh: behaviour. DE: differentially expressed.

Theme	GO ID	Gene ontology term	DE in category	Total genes in category	*p* value
np	GO:1990090	cellular response to nerve growth factor stimulus	10	131	0.0375
np	GO:0050775	positive regulation of dendrite morphogenesis	8	80	0.0399
np	GO:0048172	regulation of short-term neuronal synaptic plasticity	9	79	0.0212
np	GO:0050803	regulation of synapse structure or activity	11	72	0.0010
np	GO:1903861	positive regulation of dendrite extension	9	69	0.0330
np	GO:0051963	regulation of synapse assembly	7	54	0.0452
np	GO:0060996	dendritic spine development	5	48	0.0239
np	GO:0070983	dendrite guidance	9	30	0.0034
np	GO:0051387	negative regulation of neurotrophin TRK receptor signaling pathway	4	26	0.0410
cp	GO:2000648	positive regulation of stem cell proliferation	6	40	0.0089
cp	GO:0010458	exit from mitosis	6	39	0.0122
cp	GO:0045927	positive regulation of growth	5	25	0.0037
cp	GO:0070317	negative regulation of G0 to G1 transition	4	20	0.0209
cp	GO:0097193	intrinsic apoptotic signaling pathway	8	128	0.0225
cp	GO:0042771	intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	7	74	0.0467
cp	GO:0072577	endothelial cell apoptotic process	6	20	0.0008
cp	GO:0042981	regulation of apoptotic process	21	245	0.0013
cp	GO:0008285	negative regulation of cell proliferation	47	855	0.0325
cp	GO:0007052	mitotic spindle organization	13	181	0.0146
cp	GO:0000088	mitotic prophase	9	138	0.0327
cp	GO:0090307	mitotic spindle assembly	7	113	0.0479
cp	GO:0051225	spindle assembly	9	78	0.0086
beh	GO:0007611	learning or memory	13	138	0.0254
beh	GO:0050890	cognition	8	66	0.0374

Table 3. Selection of gene ontology (GO) categories with a significant ($p < 0.05$) overrepresentation of downregulated genes in fish exercised at volitional swimming speed, sorted by theme, i.e. np: neuroplasticity, cp: cell proliferation. DE: differentially expressed

Theme	GO ID	Gene ontology term	DE in category	Total genes in category	*p* value
np	GO:0048812	neuron projection morphogenesis	9	162	0.021
np	GO:0061001	regulation of dendritic spine morphogenesis	6	62	0.021
np	GO:0008582	regulation of synaptic growth at neuromuscular junction	4	39	0.000
np	GO:0030182	neuron differentiation	11	152	0.034
np	GO:0010976	positive regulation of neuron projection development	16	356	0.007
cp	GO:0006915	apoptotic process	64	913	0.000
cp	GO:0010940	positive regulation of necrotic cell death	5	28	0.001
cp	GO:0097193	intrinsic apoptotic signaling pathway	11	128	0.006
cp	GO:0070265	necrotic cell death	5	23	0.000
cp	GO:0008625	extrinsic apoptotic signaling pathway via death domain receptors	5	76	0.042
cp	GO:0042981	regulation of apoptotic process	14	245	0.002
cp	GO:1900740	positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	10	74	0.000
cp	GO:2001243	negative regulation of intrinsic apoptotic signaling pathway	6	60	0.018
cp	GO:0022008	neurogenesis	25	291	0.002
cp	GO:0030307	positive regulation of cell growth	13	225	0.035
cp	GO:0048680	positive regulation of axon regeneration	5	23	0.002
cp	GO:0051437	positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	24	162	0.000
cp	GO:0000090	mitotic anaphase	27	327	0.011
cp	GO:0007052	mitotic spindle organization	18	181	0.000
cp	GO:0007346	regulation of mitotic cell cycle	7	132	0.042
cp	GO:0090307	mitotic spindle assembly	10	113	0.018
cp	GO:0007088	regulation of mitotic nuclear division	7	89	0.009
cp	GO:0008156	negative regulation of DNA replication	4	37	0.047

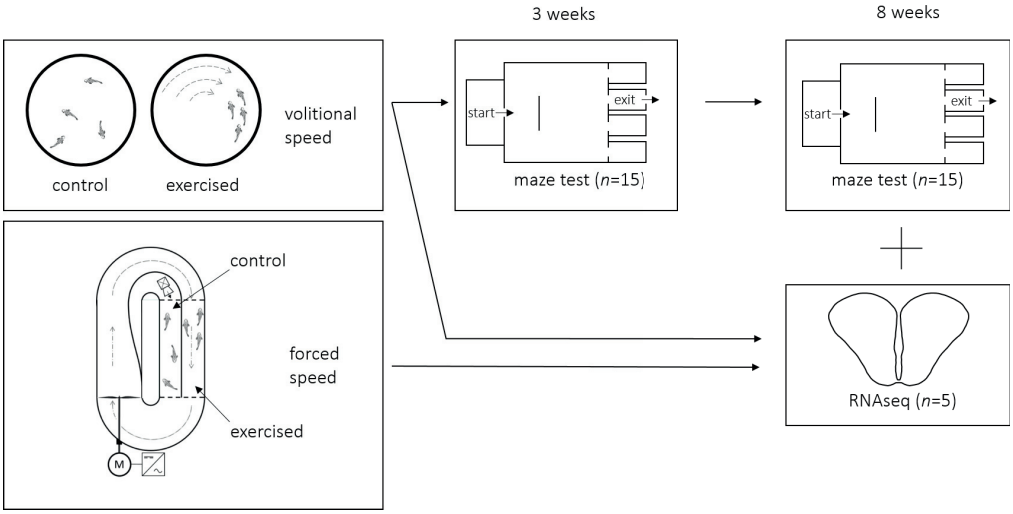
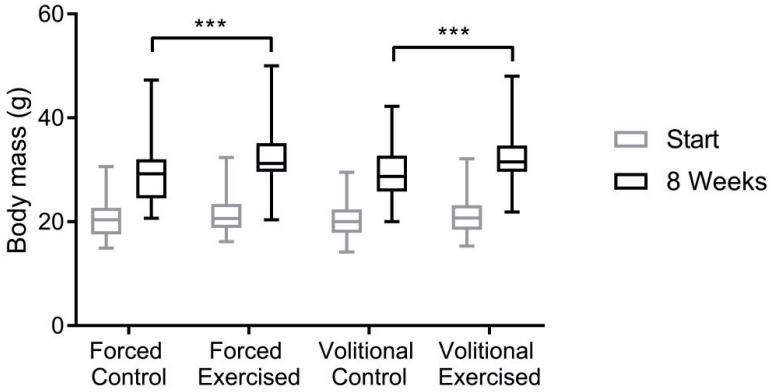
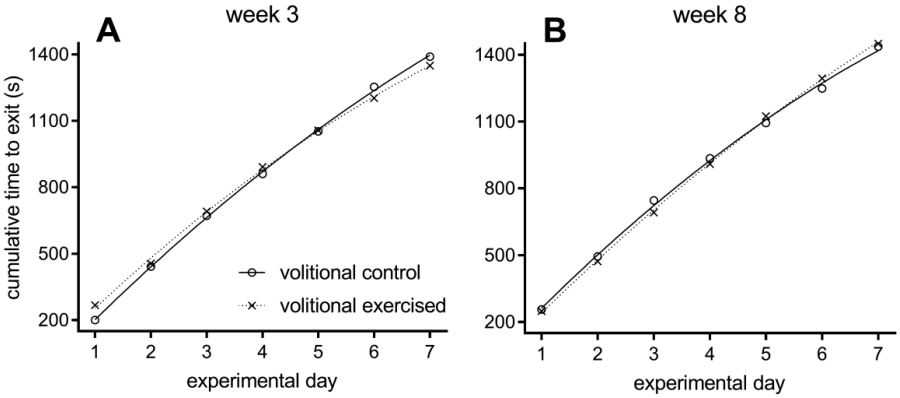


Figure 1. Overview of experimental procedures. Atlantic salmon parr were subjected to either a volitional exercise regime, where they could position themselves in currents ranging from 0.4 to 2.9 BL s^{-1} (5 – 36 cm s^{-1}), or a forced exercise regime, where they were forced to swim at a sustained speed of 3.6 BL s^{-1} (42 cm s^{-1}). Each of the treatments had a respective control group housed under identical conditions, but without increased water flow. After eight weeks of exercise treatment, the telencephalon of five fish per group (20 fish total) was sampled for RNA-seq analysis. After three and eight weeks of treatment, exercised and control fish (n = 15 per group) from the volitional treatment, were subjected to a maze test. The fish tested at eight weeks were different individuals than the fish tested at three weeks.



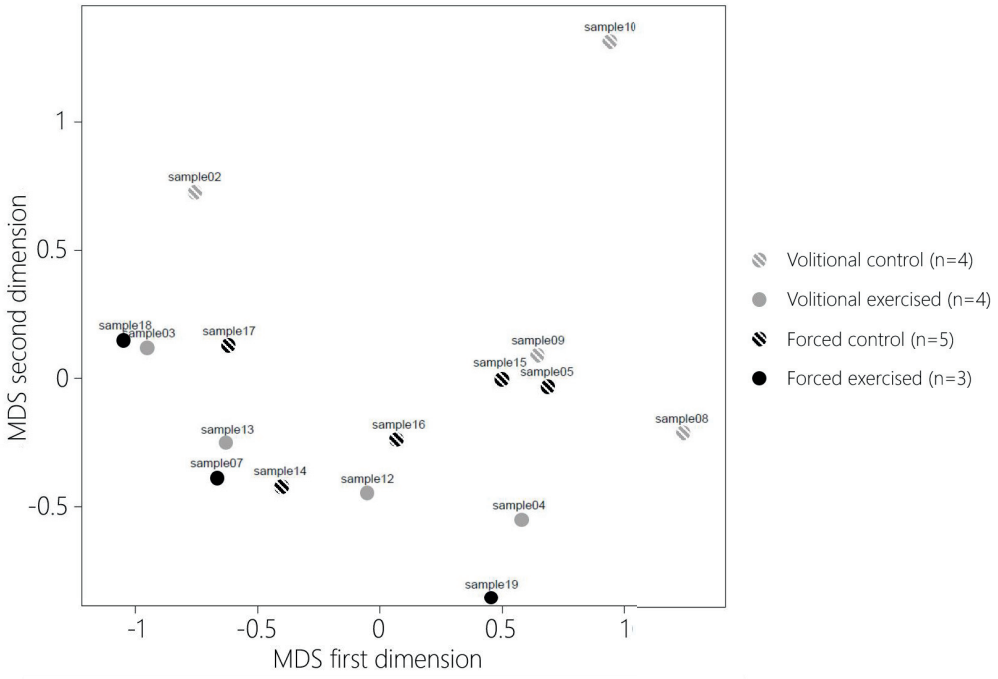
718

719 **Figure 2.** Exercise boosts growth. After eight weeks of sustained swimming, exercised fish in both the
720 volitional (W_s = 4653, Z = -3.33, p = 0.0009) and forced (W_s = 1988, Z = -3.47, p = 0.0005) exercise
721 regimes had a significantly higher body mass than control fish (Wilcoxon rank-sum test), as indicated
722 by asterisks. Centre lines within the box plots represent the median, boxes reflect quartiles, and whiskers
723 show minimum and maximum values.



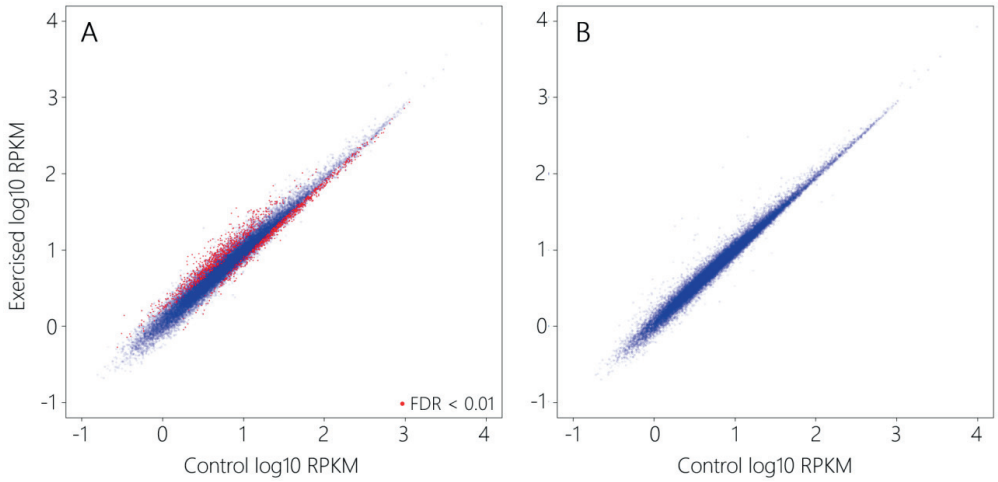
724

725 **Figure 3.** Results of the maze test. There was no difference in time to find the exit to a four-arm maze
726 between volitional exercised fish and unexercised controls, after three (**A**) and eight (**B**) weeks of
727 exercise. Lines represent model predictions based on individual data and points represent mean values.



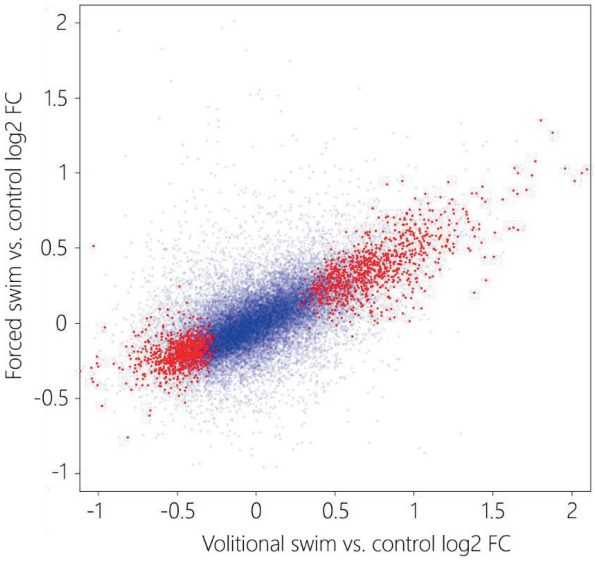
728

729 **Figure 4.** Multi-dimensional scaling (MDS) plot based on the expression of 27171 genes. The first two
730 dimensions clearly separate samples by treatment contrast (volitional exercised *vs*. volitional control,
731 and forced exercised *vs*. forced control). In addition, considering the complete dataset, the MDS plot
732 shows an almost complete division between exercised and control samples regardless of exercise
733 regime, with the exception of sample 14.



734

735 **Figure 5.** Forebrain gene expression in exercised *vs*. control salmon, subjected to either an exercise
736 regime at volitional (**A**) or forced (**B**) swimming speeds. Depicted are expression values of 27,171
737 genes, normalized for between-sample differences in sequencing depth and within-sample transcript
738 length differences (RPKM, reads per kilobase per million). Genes highlighted in red are significantly
739 differentially expressed between exercised and control groups.



740

741 **Figure 6.** Concordant changes in gene expression in volitional and forced exercised salmon. Shown is
742 the $\log_2$ fold change (FC) for each of 27,171 genes in either contrast. Genes highlighted in red are
743 significantly differentially expressed in the volitional contrast. For the vast majority of genes, fold
744 changes are similar in direction and magnitude between the treatment contrasts (Pearson correlation
745 coefficient 0.60), but none are significantly differentially expressed in the forced contrast.

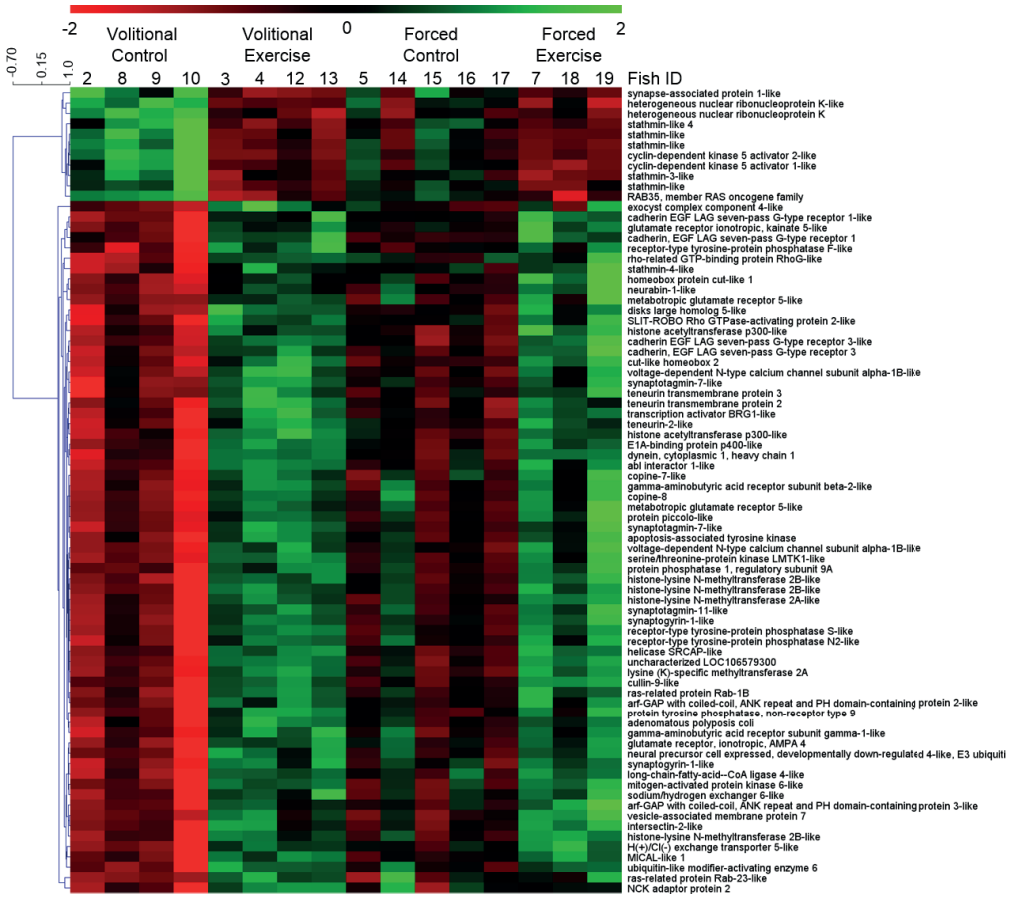


Figure 7. Heat map depicting expression of differentially expressed genes within gene ontology (GO) categories related to neuroplasticity. In order to make the absolute expression levels and amplitudes of expression changes comparable between genes, for every gene the original read per kilobase million (RPKM) values were converted to z-scores (i.e. expressed in standard deviations around the mean). Hierarchical clustering based on Pearson correlation was used to arrange genes by similarity in expression pattern. Fish ID numbers correspond to sample numbers in **Fig. 4**.

Brain training: can swimming exercise enhance brain plasticity and cognition in Atlantic salmon?

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Supplementary Material

Supplementary code

```
# Alignment and quantification, for each sample

tophat -o sample01 -i 50 -p 6 --library-type fr-unstranded --b2-very-sensitive --no-coverage-search --GTF GCF_000233375.1_ICSASG_v2_genomic_noMT.gff GCF_000233375.1_ICSASG_v2_genomic sample01.fastq.gz

samtools sort -n sample01/accepted_hits.bam sample01/accepted_hits_sorted

samtools view -h -o sample01/filtered.sam -F 0x0100 sample01/accepted_hits_sorted.bam

perl -p -i -e 's/\tNH:i:\d+/\tNH:i:1/' sample01/filtered.sam

python -m HTSeq.scripts.count -q -s no -t exon -i gene -m intersection-nonempty sample01/filtered.sam GCF_000233375.1_ICSASG_v2_genomic_noMT.gff > sample01.tsv


# Raw counts processing in R

library(edgeR)

x <- read.delim("all_data_gene_union_annotated_mrna_09042018.txt", row.names=1)


# Test only genes with a minimum number of reads

minreads <- rowMin(as.matrix(x[,2:21]))

plot(density(log10(minreads)))          # density_min_reads_12042018.pdf       # peaks at 100 reads, discard samples below 10

minreadsfilter <- minreads >=10

summary(minreadsfilter)

#             Mode   FALSE    TRUE    NA's

#          logical   21265   27171      0


xfilt <- x[minreadsfilter == TRUE,]

group <- factor(c(1,1,2,2,3,4,4,1,1,1,2,2,2,3,3,3,3,4,4,4))

design <- model.matrix(~group)

y <- DGEList(counts=xfilt[,2:21],group=group)

y <- calcNormFactors(y)

plotMDS(y)                          # MDS_edgeR_filtered_samples_12042018.pdf


# Discard outliers based on MDS and other data exploration
```

```
nooutliers <- xfilt[,1]
nooutliers <- cbind(nooutliers, xfilt[,3:6])
nooutliers <- cbind(nooutliers, xfilt[,8:11])
nooutliers <- cbind(nooutliers, xfilt[,13:20])
group <- factor(c(1,2,2,3,4,1,1,1,2,2,3,3,3,3,4,4))
y <- DGEList(counts=nooutliers[,2:17], group=group)
design <- model.matrix(~0 + group, data = y$samples)
colnames(design) <- c("vc","vs","fc","fs")
y <- calcNormFactors(y)
y$samples
#                group lib.size norm.factors
#         sample02    1 10785621    0.9863562
#         sample03    2 12870166    0.9954296
#         sample04    2 10260054    1.0002894
#         sample05    3  7177903    1.0028636
#         sample07    4 16345730    1.0030503
#         sample08    1 11907883    1.0065248
#         sample09    1 11389639    0.9984381
#         sample10    1 11779773    0.9931511
#         sample12    2 10431291    1.0096069
#         sample13    2 11756880    1.0007416
#         sample14    3 17282832    1.0038046
#         sample15    3 10580400    1.0037035
#         sample16    3 12701795    1.0017340
#         sample17    3 26983950    0.9978458
#         sample18    4 10271824    0.9943695
#         sample19    4 10724008    1.0023328

plotMDS(y)                          # MDS_edgeR_filtered_nooutliers_samples_12042018.pdf

# Differential expression testing
```

```
contrasts <- makeContrasts(vs_vc=vs-vc, fs_fc=fs-fc, fs_vs=fs-vs, fc_vc=fc-vc, levels=design)
y <- estimateDisp(y,design)
fit <- glmQLFit(y, design)
qlf_vs_vc <- glmQLFTest(fit, contrast = contrasts[,"vs_vc"])
qlf_fs_fc <- glmQLFTest(fit, contrast = contrasts[,"fs_fc"])

# Include annotations
library(Ssa.RefSeq.db)
geneid <- unique(get.id(rownames(x)))
genenames <- get.genes(geneid$gene_id, mode = "full", transcripts = "longest")
volitional <- topTags(qlf_vs_vc, n = 27171)
forced <- topTags(qlf_fs_fc, n = 27171)
cpm <- cpm(y)
alldata <- merge(genenames, cpm, by.x="gene", by.y=0)
alldata <- merge(alldata, volitional$table, by.x = "gene", by.y = 0)
alldata <- merge(alldata, forced$table, by.x = "gene", by.y = 0)
write.table(alldata, "all_data_tests_16042018.txt")                    # update with transcript lengths

# filter out duplicated genes (multiple gene <> gene_id / transcripts)
./select_unique_gene_annot.perl
alldata <- read.delim("D:/DaanMes/all_data_tests_unique_16042018.txt", header=T)

# GO testing
golist <- get.GO(alldata$gene_id, TERM="BP")
library(goseq)
volitional <- alldata$gene_id[alldata$volitional_FDR<0.01]
volitionalup <- alldata$gene_id[alldata$volitional_FDR<0.01&alldata$volitional_logFC>0]
volitionaldown <- alldata$gene_id[alldata$volitional_FDR<0.01&alldata$volitional_logFC<0]
# 1772, 923, 849

upvector <- as.integer(alldata$gene_id%in%volitionalup)
```

```
downvector <- as.integer(alldata$gene_id%in%volitionaldown)

genevector <- as.integer(alldata$gene_id%in%volitional)

names(upvector) <- alldata$gene_id

names(downvector) <- alldata$gene_id

names(genevector) <- alldata$gene_id


goall <- nullp(genevector, bias.data = alldata$transcript_length)

goalltest<- goseq(goall, gene2cat = golist, test.cats="GO:BP", method = "Wallenius")

goup <- nullp(upvector, bias.data = alldata$transcript_length)

gouptest<- goseq(goup, gene2cat = golist, test.cats="GO:BP", method = "Wallenius")

godown <- nullp(downvector, bias.data = alldata$transcript_length)

godowntest<- goseq(godown, gene2cat = golist, test.cats="GO:BP", method = "Wallenius")


# Scatterplots

png("volitional_vs_forced_scatterplot_03072018.png", width = 1000, height = 1000)

plot(alldata$volitional_logFC, alldata$forced_logFC, xlim=c(-1,2), ylim=c(-1,2), pch=20,
col="#0000aa11")

points(alldata$volitional_logFC[alldata$volitional_FDR<0.01],
alldata$forced_logFC[alldata$volitional_FDR<0.01], xlim=c(-1,2), ylim=c(-1,2), pch=20,
col="#ff0000")

dev.off()



# R version information

sessionInfo()

R version 3.4.4 (2018-03-15)

Platform: x86_64-w64-mingw32/x64 (64-bit)

Running under: Windows >= 8 x64 (build 9200)


Matrix products: default


locale:
```

[1] LC_COLLATE=English_United States.1252 LC_CTYPE=English_United States.1252

[3] LC_MONETARY=English_United States.1252 LC_NUMERIC=C

[5] LC_TIME=English_United States.1252

attached base packages:

[1] parallel stats graphics grDevices utils datasets methods base

other attached packages:

[1] goseq_1.30.0 geneLenDataBase_1.14.0 BiasedUrn_1.07 Biobase_2.38.0

[5] BiocGenerics_0.24.0 edgeR_3.20.9 limma_3.34.9 Ssa.RefSeq.db_1.2

[9] RSQLite_2.1.0

loaded via a namespace (and not attached):

[1] SummarizedExperiment_1.8.1	progress_1.1.2	locfit_1.5-9.1	splines_3.4.4
[5] lattice_0.20-35	stats4_3.4.4	rtracklayer_1.38.3	mgcv_1.8-23
[9] GenomicFeatures_1.30.3	blob_1.1.1	XML_3.98-1.10	DBI_0.8
[13] BiocParallel_1.12.0	bit64_0.9-7	matrixStats_0.53.1	GenomeInfoDbData_1.0.0
[17] stringr_1.3.0	zlibbioc_1.24.0	Biostrings_2.46.0	memoise_1.1.0
[21] IRanges_2.12.0	biomaRt_2.34.2	GenomeInfoDb_1.14.0	AnnotationDbi_1.40.0
[25] Rcpp_0.12.16	DelayedArray_0.4.1	S4Vectors_0.16.0	XVector_0.18.0
[29] bit_1.1-12	Rsamtools_1.30.0	RMySQL_0.10.14	digest_0.6.15
[33] stringi_1.1.7	GenomicRanges_1.30.3	grid_3.4.4	tools_3.4.4
[37] bitops_1.0-6	magrittr_1.5	RCurl_1.95-4.10	GO.db_3.5.0
[41] pkgconfig_2.0.1	Matrix_1.2-12	prettyunits_1.0.2	assertthat_0.2.0
[45] httr_1.3.1	R6_2.2.2	GenomicAlignments_1.14.2	nlme_3.1-131.1
[49] compiler_3.4.4			

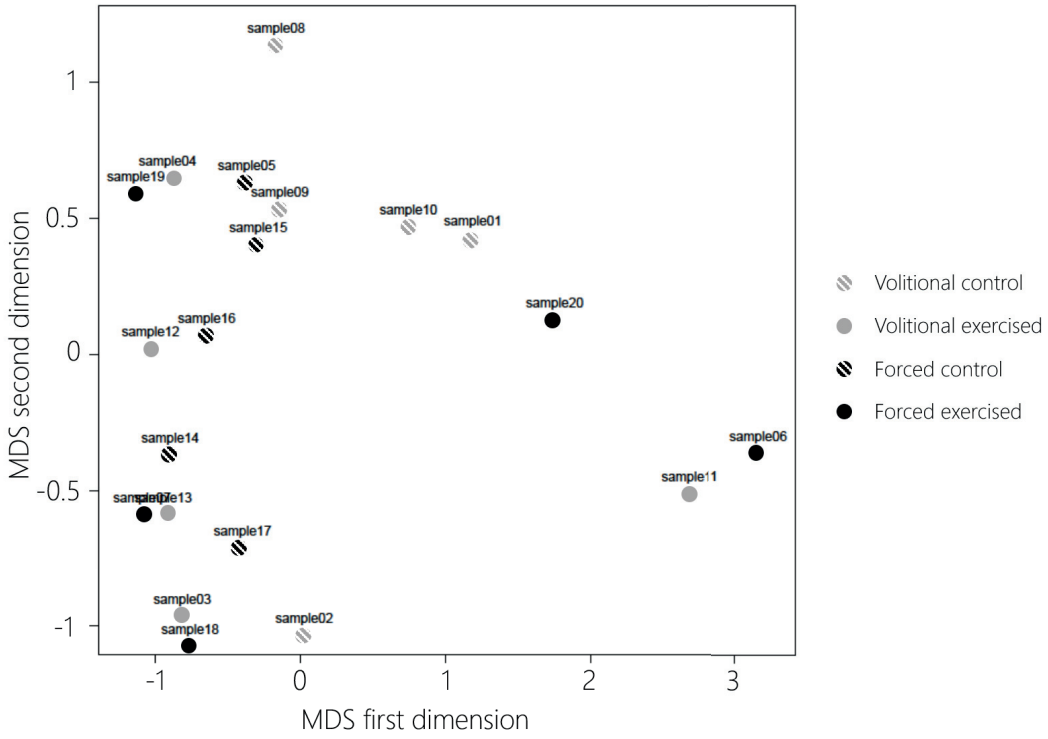

Figure S1. Initial multidimensional scaling (MDS) plot including all 20 samples, based on the expression of 27171 genes. Four samples (1, 6, 11, 20) did not cluster with their treatments and upon closer inspection, these samples were characterized by high expression of eye-related genes, suggesting a contamination with optical nerve tissue during dissection. These samples were therefore excluded from the analysis.

Table S1. Upregulated (false discovery rate (FDR) < 0.01) genes in Atlantic salmon parr subjected to 8 weeks of volitional exercise, compared to unexercised controls, expressed as fold change (FC) differences.

Gene name	Transcript ID	Product	FC
LOC106584782	XM_014170341.1	mitochondrial glutamate carrier 1-like	4.28
LOC106613326	XM_014215426.1	beta-arrestin-1	4.19
LOC106564442	XM_014130543.1	protein phosphatase 1 regulatory subunit 29-like	4.05
LOC106563916	XM_014129862.1	metabotropic glutamate receptor 5-like	3.89
LOC106590004	XM_014180484.1	AT-rich interactive domain-containing protein 4B-like	3.68
LOC106592276	XM_014183599.1	protein unc-80 homolog	3.50
LOC106611460	XM_014211674.1	E3 ubiquitin-protein ligase TRIM9-like	3.41
LOC106589110	XM_014178806.1	disks large-associated protein 3-like	3.36
LOC106610165	XM_014209350.1	extensin-1-like	3.28
LOC106611028	XM_014210842.1	endothelial PAS domain-containing protein 1-like	3.16
LOC106582526	XM_014165704.1	fibronectin-like	3.16
synj1	XM_014129816.1	synaptojanin 1	3.15
LOC106592751	XM_014184076.1	synaptotagmin-7-like	3.11
LOC106566150	XM_014134005.1	kinesin heavy chain isoform 5A-like	3.10
LOC106582460	XM_014165597.1	disintegrin and metalloproteinase domain-containing protein 23-like	3.10
LOC106563942	XM_014129897.1	phosphatidylinositol transfer protein alpha isoform-like	3.05
adcyap1r1	XM_014123308.1	adenylate cyclase activating polypeptide 1 (pituitary) receptor type I	2.96
LOC106570910	XM_014143493.1	sodium-dependent phosphate transporter 2-like	2.86
LOC106574207	XM_014149879.1	dermatopontin-like	2.84
LOC106579568	XM_014159606.1	ranBP-type and C3HC4-type zinc finger-containing protein 1-like	2.74
LOC106609628	XM_014208583.1	protein piccolo-like	2.74
LOC106592062	XM_014183365.1	caskin-1-like	2.73
LOC106589999	XM_014180480.1	AT-rich interactive domain-containing protein 4B-like	2.71
LOC106573403	XM_014148415.1	SH3 and multiple ankyrin repeat domains protein 3-like	2.70
LOC106585963	XM_014172714.1	poly [ADP-ribose] polymerase 8-like	2.69
nrsn1	XM_014181953.1	neurensin 1	2.67
LOC106572978	XM_014147574.1	neural cell adhesion molecule L1.1-like	2.67
LOC106569583	XM_014141079.1	cell adhesion molecule 3-like	2.63
LOC106586919	XM_014174667.1	myc box-dependent-interacting protein 1-like	2.61

LOC106590676	XM_014181834.1	cysteine-rich secretory protein LCCL domain-containing 1-like	2.61
LOC106605188	XM_014200603.1	protein EFR3 homolog B-like	2.60
LOC106608344	XM_014206235.1	uncharacterized LOC106608344	2.59
LOC106583499	XM_014167743.1	plasma membrane calcium-transporting ATPase 1-like	2.58
LOC106576423	XM_014153600.1	muscarinic acetylcholine receptor M2-like	2.56
LOC106569760	XM_014141350.1	rho GTPase-activating protein 29-like	2.55
LOC106609497	XM_014208374.1	muscarinic acetylcholine receptor M2-like	2.54
LOC106566945	XM_014135621.1	phosphatidylinositol 3%2C4%2C5-trisphosphate-dependent Rac exchanger 1 protein-like	2.54
LOC106581415	XM_014163473.1	metabotropic glutamate receptor 5-like	2.53
LOC106570995	XM_014143612.1	kinesin-like protein KIF3B	2.52
herc1	XM_014153804.1	HECT and RLD domain containing E3 ubiquitin protein ligase family member 1	2.48
LOC106567667	XM_014137220.1	protein phosphatase 1E-like	2.43
chst12	XM_014186112.1	carbohydrate (chondroitin 4) sulfotransferase 12	2.41
LOC106608754	XM_014206871.1	vigilin-like	2.39
pdcl	XM_014123682.1	phosducin-like	2.39
LOC106595266	XM_014186640.1	glutamate receptor 2-like	2.38
LOC106562493	XM_014127402.1	microtubule-associated protein 1A-like	2.38
igf1r	XM_014124119.1	insulin-like growth factor 1 receptor	2.38
LOC106601049	XM_014192927.1	cAMP-specific 3'%2C5'-cyclic phosphodiesterase 4D-like	2.37
LOC106573449	XM_014148501.1	protein piccolo-like	2.37
LOC106595230	XM_014186610.1	neural cell adhesion molecule L1-like	2.35
LOC106566388	XM_014134363.1	sodium-dependent neutral amino acid transporter SLC6A17-like	2.34
LOC106589899	XM_014180316.1	serine/threonine-protein kinase LMTK1-like	2.34
LOC106611901	XM_014212556.1	janus kinase and microtubule-interacting protein 1-like	2.34
vgll4	XM_014168352.1	vestigial-like family member 4	2.34
LOC106572946	XM_014147537.1	IQ motif and SEC7 domain-containing protein 2-like	2.34
LOC106560380	XM_014123252.1	rho GTPase-activating protein 39-like	2.33
LOC106604362	XM_014198918.1	monocarboxylate transporter 8-like	2.32
LOC106568697	XM_014139263.1	abl interactor 1-like	2.32
LOC106567148	XM_014136111.1	basement membrane-specific heparan sulfate proteoglycan core protein-like	2.32
LOC106560445	XM_014123362.1	breast cancer metastasis-suppressor 1 homolog	2.30
LOC106606938	XM_014203427.1	glycine receptor subunit beta-like	2.30
slc6a11	XM_014168361.1	solute carrier family 6 (neurotransmitter transporter)%2C member 11	2.30

LOC106576213	XM_014153216.1	voltage-dependent P/Q-type calcium channel subunit alpha-1A-like	2.30
LOC106603846	XM_014198033.1	gamma-aminobutyric acid receptor subunit beta-2-like	2.26
cssa09h20orf194	XM_014211656.1	chromosome ssa09 open reading frame%2C human C20orf194	2.25
LOC106578942	XM_014158231.1	neurexin-1a-like	2.25
LOC106565592	XM_014132881.1	disco-interacting protein 2 homolog B-A-like	2.25
hnrnpul1	XM_014215521.1	heterogeneous nuclear ribonucleoprotein U-like 1	2.25
LOC106581527	XM_014163631.1	ubiquitin carboxyl-terminal hydrolase 32-like	2.25
LOC106570681	XM_014143154.1	gamma-aminobutyric acid type B receptor subunit 1-like	2.24
LOC106578054	XM_014156620.1	NHS-like protein 2	2.24
LOC106593073	XM_014184416.1	partner and localizer of BRCA2-like	2.24
LOC106613305	XM_014215393.1	neprilysin-like	2.23
LOC106570801	XM_014143337.1	calcium/calmodulin-dependent protein kinase type II subunit beta-like	2.23
LOC106573868	XM_014149278.1	probable cation-transporting ATPase 13A3	2.23
LOC106592932	XM_014184288.1	PHD finger protein 21B-like	2.21
LOC106607452	XM_014204412.1	synaptogyrin-1-like	2.21
LOC106563878	XM_014129802.1	tripartite motif-containing protein 3-like	2.20
LOC106612202	XM_014213167.1	leucine-rich repeat extensin-like protein 5	2.20
LOC106563893	XM_014129830.1	splicing factor%2C arginine/serine-rich 15-like	2.19
LOC106605517	XM_014201257.1	sodium- and chloride-dependent transporter XTRP3-like	2.17
LOC106586624	XM_014174111.1	immunoglobulin superfamily member 3-like	2.17
LOC106561392	XM_014125320.1	homeodomain-interacting protein kinase 2-like	2.17
LOC106605888	XM_014201881.1	uncharacterized protein DDB_G0284459-like	2.16
LOC106591965	XM_014183239.1	serine/threonine-protein kinase DCLK1-like	2.15
LOC106571967	XM_014145585.1	death-inducer obliterator 1-like	2.15
LOC106611279	XM_014211318.1	tau-tubulin kinase 2-like	2.15
LOC106579599	XM_014159658.1	histone-lysine N-methyltransferase 2C-like	2.14
LOC106610406	XM_014209771.1	circadian locomoter output cycles protein kaput-like	2.14
LOC106574702	XM_014150714.1	striated muscle preferentially expressed protein kinase-like	2.13
LOC106561741	XM_014125968.1	pleckstrin homology domain-containing family A member 5-like	2.13
LOC106582632	XM_014165868.1	chromodomain-helicase-DNA-binding protein 6-like	2.13
LOC106572123	XM_014145993.1	WD repeat-containing protein 7-like	2.12
LOC106588753	XM_014178093.1	F-box/LRR-repeat protein 2-like	2.12
LOC106572850	XM_014147383.1	monocarboxylate transporter 5-like	2.12

LOC106612075	XM_014212904.1	ATP-binding cassette sub-family A member 5-like	2.12
LOC106609808	XM_014208831.1	probable E3 ubiquitin-protein ligase makorin-1	2.11
LOC106580977	XM_014162587.1	neurobeachin-like	2.11
pvrl1	NM_001146661.1	Poliovirus receptor-related protein 1	2.11
LOC106563271	XM_014128714.1	zinc finger protein 618-like	2.11
LOC106594246	XM_014185622.1	trinucleotide repeat-containing gene 18 protein-like	2.11
LOC106600127	XM_014191463.1	tensin-1-like	2.11
LOC106562976	XM_014128136.1	disabled homolog 2-interacting protein-like	2.10
tmem55a	XM_014178844.1	transmembrane protein 55A	2.10
LOC106591642	XM_014182848.1	fascin-like	2.10
LOC106580646	XM_014161925.1	leucine-rich repeat and calponin homology domain-containing protein 3-like	2.09
LOC106583361	XM_014167493.1	ERC protein 2-like	2.09
LOC106573711	XM_014149012.1	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2%2C3-sialyltransferase 2-like	2.09
LOC106603714	XM_014197733.1	calpain-5-like	2.09
cdk19	XM_014144231.1	cyclin-dependent kinase 19	2.09
LOC106580423	XM_014161465.1	clathrin heavy chain 1-like	2.09
LOC106572920	XM_014147506.1	methyl-CpG-binding protein 2-like	2.09
LOC106580500	XM_014161647.1	phosphatidate cytidylyltransferase 2-like	2.08
LOC106572669	XM_014147046.1	histone-lysine N-methyltransferase 2D-like	2.08
LOC106595370	XM_014186743.1	fascin-like	2.07
sergef	XM_014126054.1	secretion regulating guanine nucleotide exchange factor	2.07
LOC106613358	XM_014215481.1	ubiquitin carboxyl-terminal hydrolase 32-like	2.07
LOC106572749	XM_014147193.1	tensin-2-like	2.07
LOC106575448	XM_014151948.1	mucin-17-like	2.07
LOC106585059	XM_014170819.1	clathrin heavy chain 1-like	2.06
LOC106578744	XM_014157877.1	lysosomal-trafficking regulator-like	2.06
LOC106569883	XM_014141596.1	synaptotagmin-11-like	2.06
LOC106566514	XM_014134609.1	ankyrin-1-like	2.06
LOC106595312	XM_014186688.1	putative ubiquitin carboxyl-terminal hydrolase 50	2.06
LOC106565257	XM_014132162.1	plexin-B1-like	2.05
LOC106608262	XM_014206116.1	synapse differentiation-inducing gene protein 1-like	2.05
LOC106570191	XM_014142238.1	ras/Rap GTPase-activating protein SynGAP-like	2.05
LOC106601156	XM_014193116.1	glutamate receptor ionotropic%2C NMDA 2B-like	2.05

LOC106562562	XM_014127503.1	uncharacterized protein KIAA0895-like	2.05
LOC106582115	XM_014164868.1	nectin-4-like	2.05
LOC106610931	XM_014210616.1	gamma-aminobutyric acid receptor subunit gamma-1-like	2.04
LOC106611402	XM_014211572.1	protein Jade-1-like	2.03
LOC106569696	XM_014141259.1	receptor-type tyrosine-protein phosphatase F-like	2.03
LOC106609939	XM_014208975.1	protocadherin Fat 1-like	2.03
LOC106580561	XM_014161750.1	protein patched homolog 1-like	2.03
LOC100380784	XM_014146808.1	basement membrane-specific heparan sulfate proteoglycan core protein	2.02
LOC106567317	XM_014136454.1	neural proliferation differentiation and control protein 1-like	2.02
LOC106577760	XM_014156083.1	PERQ amino acid-rich with GYF domain-containing protein 1-like	2.02
LOC106568900	XM_014139690.1	lipid phosphate phosphatase-related protein type 4-like	2.02
LOC106579300	XM_014159100.1	uncharacterized LOC106579300	2.01
LOC106606600	XM_014202906.1	trinucleotide repeat-containing gene 6C protein-like	2.01
LOC106579925	XM_014160329.1	cationic amino acid transporter 4-like	2.01
vps13a	XM_014172179.1	vacuolar protein sorting 13 homolog A (S. cerevisiae)	2.01
LOC106582171	XM_014164978.1	A-kinase anchor protein 11-like	2.01
LOC106609034	XM_014207368.1	wiskott-Aldrich syndrome protein family member 3-like	2.01
LOC106568132	XM_014138176.1	ras-specific guanine nucleotide-releasing factor 2-like	2.01
LOC106572720	XM_014147149.1	uncharacterized LOC106572720	2.00
LOC106613214	XM_014215254.1	cytoplasmic dynein 2 heavy chain 1-like	2.00
LOC106589521	XM_014179606.1	DNA-directed RNA polymerase III subunit RPC5-like	2.00
LOC106575544	XM_014152103.1	tensin-1-like	2.00
LOC106584219	XM_014169258.1	cytoplasmic polyadenylation element-binding protein 2-like	2.00
LOC106566329	XM_014134243.1	synaptophysin-like	1.99
LOC106571269	XM_014144096.1	collagen alpha-1(XII) chain-like	1.99
nkcc1a	NM_001123683.1	Na/K/2Cl co-transporter	1.99
LOC106584716	XM_014170233.1	ubiquitin-conjugating enzyme E2 Q2-like	1.99
sobp	XM_014192170.1	sine oculis binding protein homolog	1.99
cux2	XM_014160397.1	cut-like homeobox 2	1.99
LOC106570633	XM_014143099.1	F-box/LRR-repeat protein 2-like	1.98
LOC106575743	XM_014152416.1	protein unc-80 homolog	1.98
LOC106585713	XM_014172239.1	cytoplasmic phosphatidylinositol transfer protein 1-like	1.98
LOC106607176	XM_014203842.1	histone deacetylase 5-like	1.97

LOC106583168	XM_014167035.1	electroneutral sodium bicarbonate exchanger 1-like	1.97
LOC106590617	XM_014181747.1	beta-1%2C4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase-like	1.97
LOC106565730	XM_014133161.1	contactin-3-like	1.97
syt17	XM_014194413.1	synaptotagmin XVII	1.97
gfod1	XM_014181121.1	glucose-fructose oxidoreductase domain containing 1	1.97
LOC106565081	XM_014131720.1	calmodulin-binding transcription activator 2-like	1.97
LOC106611429	XM_014211625.1	glioma tumor suppressor candidate region gene 1 protein-like	1.97
LOC106594098	XM_014185469.1	shaker-related potassium channel tsha2-like	1.96
LOC106562829	XM_014127851.1	ral guanine nucleotide dissociation stimulator-like	1.96
LOC106601859	XM_014194274.1	uncharacterized serine-rich protein C215.13-like	1.96
LOC100380696	XM_014152358.1	fibronectin	1.96
LOC106605949	XM_014201961.1	tyrosine-protein phosphatase non-receptor type 3-like	1.96
LOC106591034	XM_014182204.1	glycogenin-1-like	1.96
LOC106582279	XM_014165186.1	leishmanolysin-like peptidase	1.96
ptch1	XM_014171042.1	patched 1	1.96
LOC106570525	XM_014142941.1	juxtaposed with another zinc finger protein 1	1.96
LOC106609675	XM_014208646.1	exocyst complex component 4-like	1.95
LOC106582221	XM_014165078.1	A-kinase anchor protein 11-like	1.95
btbd7	XM_014145062.1	BTB (POZ) domain containing 7	1.95
LOC106563134	XM_014128379.1	voltage-dependent N-type calcium channel subunit alpha-1B-like	1.95
LOC106573195	XM_014147980.1	solute carrier family 45 member 3-like	1.95
LOC106576414	XM_014153573.1	POU domain%2C class 2%2C transcription factor 2-like	1.94
LOC106603934	XM_014198188.1	calcium/calmodulin-dependent protein kinase kinase 1-like	1.94
LOC106611949	XM_014212651.1	A disintegrin and metalloproteinase with thrombospondin motifs 2-like	1.94
LOC106573269	XM_014148178.1	UPF0606 protein KIAA1549-like	1.94
LOC106599334	XM_014190509.1	sickle tail protein homolog	1.94
LOC106566796	XM_014135196.1	protein SOGA1-like	1.94
LOC106571799	XM_014145259.1	forkhead box protein O3-like	1.94
LOC106610770	XM_014210326.1	filamin-A-interacting protein 1-like	1.94
dock5	XM_014160188.1	dedicator of cytokinesis 5	1.94
LOC106569531	XM_014140980.1	transportin-1-like	1.93
LOC106612174	XM_014213111.1	rho guanine nucleotide exchange factor 9	1.93
acacb	XM_014160782.1	acetyl-CoA carboxylase beta	1.93

LOC106578063	XM_014156632.1	serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit beta isoform-like	1.93
LOC106605289	XM_014200762.1	OTU domain-containing protein 7B-like	1.93
LOC106577167	XM_014154979.1	tau-tubulin kinase 1-like	1.92
LOC106568602	XM_014139059.1	disabled homolog 2-interacting protein-like	1.92
LOC106574153	XM_014149798.1	receptor-type tyrosine-protein phosphatase S-like	1.92
LOC106611865	XM_014212478.1	A disintegrin and metalloproteinase with thrombospondin motifs 2-like	1.92
LOC106579284	XM_014159065.1	uncharacterized LOC106579284	1.92
LOC106570613	XM_014143076.1	probable serine/threonine-protein kinase kinX	1.91
LOC106575851	XM_014152553.1	Nance-Horan syndrome protein-like	1.91
LOC106577504	XM_014155564.1	ankyrin-2-like	1.91
LOC106568281	XM_014138468.1	phosphatidylinositol 4-kinase alpha-like	1.91
LOC106560483	XM_014123444.1	dedicator of cytokinesis protein 7-like	1.91
LOC106583531	XM_014167828.1	calcium/calmodulin-dependent protein kinase type 1-like	1.91
LOC106568312	XM_014138534.1	membrane-associated phosphatidylinositol transfer protein 2-like	1.91
LOC106568400	XM_014138709.1	integrin alpha-1-like	1.91
LOC106573992	XM_014149476.1	cyclin-dependent kinase-like 5	1.90
LOC106578941	XM_014158229.1	neurexin-1a-beta-like	1.90
LOC106585259	XM_014171270.1	ankyrin-1-like	1.90
zfhx3	XM_014126638.1	zinc finger homeobox 3	1.90
LOC106574979	XM_014151114.1	dystrophin-like	1.90
LOC106591047	XM_014182234.1	kinesin-associated protein 3-like	1.89
LOC106597634	XM_014188796.1	tetratricopeptide repeat protein 17-like	1.89
LOC106573459	XM_014148523.1	FERM domain-containing protein 4A-like	1.89
clock	XM_014169007.1	clock circadian regulator	1.89
LOC106570447	XM_014142790.1	gamma-aminobutyric acid type B receptor subunit 2-like	1.89
kmt2d	XM_014135873.1	lysine (K)-specific methyltransferase 2D	1.89
LOC106605889	XM_014201882.1	RUN and SH3 domain-containing protein 1-like	1.89
chd3	XM_014155949.1	chromodomain helicase DNA binding protein 3	1.88
LOC106608439	XM_014206372.1	palmitoyltransferase ZDHHC3-like	1.88
LOC106602308	XM_014194858.1	MAX gene-associated protein-like	1.88
LOC106568226	XM_014138375.1	lysine-specific demethylase 2B-like	1.88
rnf123	XM_014168343.1	ring finger protein 123	1.88
LOC106578973	XM_014158322.1	serine-rich coiled-coil domain-containing protein 2-like	1.88

LOC106610487	XM_014209867.1	protein TANC2-like	1.88
LOC106592162	XM_014183471.1	insulin-like growth factor 1 receptor	1.88
LOC106576674	XM_014153915.1	neuronal cell adhesion molecule-like	1.88
LOC106572064	XM_014145841.1	protein 4.1-like	1.88
LOC106572368	XM_014146445.1	plexin-A1-like	1.87
LOC106580439	XM_014161499.1	histone-lysine N-methyltransferase NSD3-like	1.87
ralgapa2	XM_014210399.1	Ral GTPase activating protein%2C alpha subunit 2 (catalytic)	1.87
LOC106586920	XM_014174668.1	translation initiation factor IF-2-like	1.87
cd164	XM_014205492.1	CD164 molecule%2C sialomucin	1.87
LOC106563815	XM_014129707.1	rapamycin-insensitive companion of mTOR-like	1.86
LOC106594999	XM_014186381.1	protein tweety homolog 3-like	1.86
LOC106586931	XM_014174683.1	neuropilin-2-like	1.86
LOC106563781	XM_014129632.1	protein furry homolog	1.86
LOC106613701	XM_014216233.1	excitatory amino acid transporter 1-like	1.86
LOC106612815	XM_014214353.1	ras-related protein Rab-23-like	1.86
LOC106585272	XM_014171314.1	membrane-associated phosphatidylinositol transfer protein 2-like	1.85
LOC106569478	XM_014140866.1	uncharacterized LOC106569478	1.85
fbxl16	XM_014202492.1	F-box and leucine-rich repeat protein 16	1.85
LOC106612058	XM_014212875.1	LON peptidase N-terminal domain and RING finger protein 1-like	1.85
LOC106562618	XM_014127575.1	CREB-regulated transcription coactivator 3-like	1.85
LOC106578893	XM_014158131.1	uncharacterized protein KIAA0195-like	1.85
cbl	XM_014213633.1	Cbl proto-oncogene%2C E3 ubiquitin protein ligase	1.85
LOC106569805	XM_014141435.1	probable helicase senataxin	1.85
LOC106601252	XM_014193315.1	histone deacetylase 5-like	1.84
LOC106570463	XM_014142825.1	neurabin-1-like	1.84
LOC106569018	XM_014139973.1	kinesin-like protein KIF26B	1.84
vdr0	XM_014146371.1	vitamin D receptor	1.84
LOC106609237	XM_014207789.1	striatin-interacting protein 1 homolog	1.84
LOC106611507	XM_014211774.1	protein diaphanous homolog 2-like	1.84
LOC106571009	XM_014143627.1	myotubularin-related protein 9-like	1.83
LOC106574299	XM_014150103.1	potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 3-like	1.83
LOC106575892	XM_014152608.1	protein FAM117B-like	1.83
LOC106571361	XM_014144366.1	A-kinase anchor protein 7 isoform gamma-like	1.83

LOC106613636	XM_014216094.1	microtubule-associated serine/threonine-protein kinase 3-like	1.83
LOC106568027	XM_014138028.1	homeobox protein cut-like 1	1.83
LOC106568376	XM_014138668.1	rab GTPase-activating protein 1-like	1.83
LOC106594565	XM_014185929.1	formin-2-like	1.83
rbm33	XM_014180672.1	RNA binding motif protein 33	1.83
edc4	XM_014149081.1	enhancer of mRNA decapping 4	1.83
LOC106573085	XM_014147770.1	contactin-1a-like	1.82
LOC106591933	XM_014183199.1	oxysterol-binding protein-related protein 3-like	1.82
LOC106605998	XM_014202048.1	nibrin-like	1.82
LOC106595407	XM_014186778.1	casein kinase I isoform delta-B-like	1.82
LOC106585658	XM_014172094.1	acetyl-CoA carboxylase 2-like	1.81
LOC106592729	XM_014184055.1	GPI transamidase component PIG-S-like	1.81
rapgef2	XM_014199228.1	Rap guanine nucleotide exchange factor (GEF) 2	1.81
aplp2	XM_014215554.1	amyloid beta (A4) precursor-like protein 2	1.81
LOC106606311	XM_014202467.1	charged multivesicular body protein 6-like	1.81
LOC106588669	XM_014177879.1	thyroid hormone receptor beta	1.81
wdr11	XM_014154429.1	WD repeat domain 11	1.80
dag1	XM_014168313.1	dystroglycan 1 (dystrophin-associated glycoprotein 1)	1.80
LOC106583703	XM_014168207.1	glycerol-3-phosphate dehydrogenase [NAD(+)]%2C cytoplasmic-like	1.80
LOC106590861	XM_014182039.1	leucine-rich repeat extensin-like protein 1	1.80
LOC106583566	XM_014167901.1	CUGBP Elav-like family member 4	1.80
LOC106606768	XM_014203126.1	voltage-dependent P/Q-type calcium channel subunit alpha-1A-like	1.80
pcdh9	XM_014151127.1	protocadherin 9	1.80
ptprr	XM_014208833.1	protein tyrosine phosphatase%2C receptor type%2C R	1.79
lrch3	XM_014213547.1	leucine-rich repeats and calponin homology (CH) domain containing 3	1.79
LOC106612514	XM_014213716.1	alpha-2-macroglobulin-like	1.79
LOC106606599	XM_014202904.1	retinoic acid-induced protein 1-like	1.79
LOC106566323	XM_014134234.1	CXXC-type zinc finger protein 1-like	1.79
LOC106572809	XM_014147301.1	tumor necrosis factor receptor superfamily member 5-like	1.79
nedd4l	XM_014138852.1	neural precursor cell expressed%2C developmentally down-regulated 4-like%2C E3 ubiquitin protein ligase	1.79
LOC106585249	XM_014171260.1	phosphatidate cytidylyltransferase 2-like	1.79
LOC106562089	XM_014126655.1	copine-7-like	1.79

ddx6	XM_014215579.1	DEAD (Asp-Glu-Ala-Asp) box helicase 6	1.79
LOC106597569	XM_014188737.1	protein 4.1 homolog	1.78
LOC106578174	XM_014156806.1	tripartite motif-containing protein 2-like	1.78
LOC106595470	XM_014186843.1	serine/threonine-protein kinase PAK 2-like	1.78
LOC106583746	XM_014168288.1	dedicator of cytokinesis protein 3-like	1.78
LOC106590738	XM_014181898.1	beta-1%2C4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase-like	1.78
LOC106586786	XM_014174419.1	peripheral plasma membrane protein CASK-like	1.78
LOC106575987	XM_014152769.1	copine-8	1.78
LOC106586946	XM_014174704.1	ras-associated and pleckstrin homology domains-containing protein 1-like	1.78
nphp4	XM_014135428.1	nephronophthisis 4	1.78
LOC106611270	XM_014211303.1	endoribonuclease Dicer-like	1.78
LOC106574143	XM_014149776.1	guanine nucleotide-binding protein subunit beta-4	1.78
LOC106611370	XM_014211528.1	neurexin-3a-like	1.78
LOC106603820	XM_014197991.1	folliculin-interacting protein 1-like	1.78
LOC106560530	XM_014123504.1	microtubule-associated serine/threonine-protein kinase 2-like	1.77
LOC106602516	XM_014195192.1	ubiquitin-like modifier-activating enzyme 6	1.77
LOC106588723	XM_014177998.1	uncharacterized LOC106588723	1.77
LOC106589430	XM_014179414.1	neurotrypsin-like	1.77
LOC106582455	XM_014165579.1	dedicator of cytokinesis protein 9-like	1.77
LOC106610776	XM_014210337.1	disks large-associated protein 2-like	1.77
LOC106564132	XM_014130147.1	lysosomal alpha-glucosidase-like	1.77
LOC106604864	XM_014199940.1	janus kinase and microtubule-interacting protein 1-like	1.77
LOC106583767	XM_014168326.1	homeodomain-interacting protein kinase 1-like	1.77
slc7a3	XM_014199635.1	solute carrier family 7 (cationic amino acid transporter%2C y+ system)%2C member 3	1.77
LOC106599465	XM_014190712.1	nuclear receptor coactivator 2-like	1.76
LOC106586426	XM_014173671.1	CLIP-associating protein 1-B-like	1.76
LOC106591770	XM_014182978.1	trinucleotide repeat-containing gene 18 protein-like	1.76
LOC106613097	XM_014215015.1	unconventional myosin-XVIIIa-like	1.76
LOC106610979	XM_014210738.1	leucine-rich repeat-containing protein 9-like	1.76
LOC106573256	XM_014148161.1	retinoblastoma-like protein 2	1.76
LOC106566728	XM_014135048.1	nuclear receptor coactivator 3-like	1.76
sfmbt2	XM_014207878.1	Scm-like with four mbt domains 2	1.76
LOC106583686	XM_014168179.1	proto-oncogene tyrosine-protein kinase Src-like	1.75

i2c3	XM_014176828.1	Eukaryotic translation initiation factor 2C 3	1.75
LOC106574911	XM_014151046.1	multiple epidermal growth factor-like domains protein 8	1.75
LOC106561324	XM_014125162.1	serine/threonine-protein kinase WNK1-like	1.75
otof	XM_014145198.1	otoferlin	1.75
micall1	XM_014130683.1	MICAL-like 1	1.75
LOC106572997	XM_014147591.1	potassium voltage-gated channel subfamily A member 2-like	1.75
LOC106593268	XM_014184606.1	protein flightless-1 homolog	1.75
LOC106584443	XM_014169785.1	CUGBP Elav-like family member 5	1.74
LOC106577394	XM_014155365.1	acyl-CoA desaturase-like	1.74
LOC106588748	XM_014178086.1	upstream-binding protein 1-like	1.74
LOC106568585	XM_014139033.1	uncharacterized LOC106568585	1.74
LOC106561729	XM_014125948.1	tumor protein p53-inducible protein 11-like	1.73
LOC106563093	XM_014128322.1	uncharacterized LOC106563093	1.73
LOC106584763	XM_014170311.1	potassium voltage-gated channel subfamily C member 1-like	1.73
LOC106565077	XM_014131710.1	solute carrier family 12 member 5-like	1.73
LOC106588140	XM_014176832.1	aquaporin-3-like	1.73
LOC106562412	XM_014127283.1	membrane-associated phosphatidylinositol transfer protein 2-like	1.73
LOC106566892	XM_014135492.1	disintegrin and metalloproteinase domain-containing protein 17-like	1.73
LOC106607841	XM_014205259.1	protein ZNF365-like	1.73
LOC106603484	XM_014197244.1	proline-rich protein 7-like	1.73
LOC106577281	XM_014155222.1	uncharacterized LOC106577281	1.73
ahr2b	NM_001123556.1	aryl hydrocarbon receptor 2 beta	1.73
LOC106589035	XM_014178683.1	eyes absent homolog 3-like	1.73
LOC106575885	XM_014152596.1	gastrula zinc finger protein XlCGF17.1-like	1.73
LOC106588641	XM_014177818.1	transcription factor HIVEP3-like	1.72
LOC106572628	XM_014146986.1	serine/threonine-protein phosphatase 5-like	1.72
LOC106611291	XM_014211337.1	pleckstrin homology domain-containing family H member 1-like	1.72
LOC106560750	XM_014123959.1	zinc finger protein 609-like	1.72
cnnm3	XM_014128009.1	cyclin and CBS domain divalent metal cation transport mediator 3	1.72
LOC106589275	XM_014179026.1	helicase SRCAP-like	1.72
LOC106589506	XM_014179577.1	serine/threonine-protein kinase SBK1-like	1.72
LOC106574855	XM_014150973.1	R3H domain-containing protein 1-like	1.72
LOC106588045	XM_014176710.1	low-density lipoprotein receptor-related protein 3-like	1.71

LOC106565713	XM_014133120.1	SLIT-ROBO Rho GTPase-activating protein 2-like	1.71
LOC106568675	XM_014139196.1	serine/threonine-protein kinase PRP4 homolog	1.71
LOC106613037	XM_014214916.1	serine/threonine-protein kinase TAO1-like	1.71
LOC106566447	XM_014134494.1	syntaphilin-like	1.71
hdac4	XM_014164292.1	histone deacetylase 4	1.71
stxbp5	XM_014145279.1	syntaxin binding protein 5 (tomosyn)	1.71
zcchc8	XM_014161314.1	zinc finger%2C CCHC domain containing 8	1.71
LOC106607600	XM_014204689.1	adenosine 3'-phospho 5'-phosphosulfate transporter 1-like	1.71
nck2	XM_014174379.1	NCK adaptor protein 2	1.71
arfgef2	XM_014145961.1	ADP-ribosylation factor guanine nucleotide-exchange factor 2 (brefeldin A-inhibited)	1.71
LOC106583942	XM_014168640.1	tumor suppressor candidate 5 homolog	1.70
LOC106565299	XM_014132244.1	voltage-dependent L-type calcium channel subunit alpha-1D-like	1.70
LOC106562309	XM_014127101.1	protein lin-54 homolog	1.70
LOC106579370	XM_014159210.1	protein phosphatase 1 regulatory subunit 1B-like	1.70
LOC106606041	XM_014202115.1	kinesin-like protein KIF13A	1.70
LOC106603105	XM_014196330.1	leucine-rich repeat and calponin homology domain-containing protein 2-like	1.70
LOC106588883	XM_014178393.1	inactive phospholipase C-like protein 2	1.70
LOC106602439	XM_014195080.1	protein WWC2-like	1.69
LOC106567020	XM_014135795.1	serine/threonine-protein kinase WNK2-like	1.69
LOC106567401	XM_014136584.1	peripheral-type benzodiazepine receptor-associated protein 1-like	1.69
LOC106601438	XM_014193653.1	RNA binding protein fox-1 homolog 3-like	1.69
LOC106601595	XM_014193918.1	dynamin-2-like	1.69
LOC106608994	XM_014207312.1	phosphofurin acidic cluster sorting protein 1-like	1.69
LOC106573061	XM_014147715.1	CUGBP Elav-like family member 2	1.69
LOC106580493	XM_014161626.1	methylcytosine dioxygenase TET3-like	1.69
LOC106604912	XM_014200037.1	ras-GEF domain-containing family member 1C-like	1.69
pusl1	XM_014168350.1	pseudouridylate synthase-like 1	1.69
LOC106602984	XM_014196077.1	pecanex-like protein 1	1.69
LOC106567492	XM_014136782.1	glucocorticoid receptor-like	1.69
LOC106604036	XM_014198371.1	B-cell receptor CD22-like	1.69
LOC106610280	XM_014209542.1	general vesicular transport factor p115-like	1.68
fbxo15	XM_014157193.1	F-box protein 15	1.68
vps39	XM_014211709.1	vacuolar protein sorting 39 homolog (S. cerevisiae)	1.68

LOC106610942	XM_014210634.1	uncharacterized protein KIAA1109-like	1.68
LOC106570883	XM_014143453.1	DENN domain-containing protein 1A-like	1.68
LOC106606288	XM_014202432.1	zinc finger protein 239-like	1.68
LOC106577545	XM_014155660.1	equilibrative nucleoside transporter 2-like	1.68
LOC106580373	XM_014161353.1	probable E3 ubiquitin-protein ligase HECTD4	1.68
LOC106560529	XM_014123503.1	phosphatidylinositol 3-kinase regulatory subunit gamma-like	1.68
LOC106590429	XM_014181455.1	histone-lysine N-methyltransferase 2C-like	1.67
LOC106587746	XM_014176363.1	inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase 2-like	1.67
LOC106587514	XM_014175965.1	synaptotagmin-7-like	1.67
pkdcc	XM_014201656.1	protein kinase domain containing%2C cytoplasmic	1.67
papolg	XM_014216469.1	poly(A) polymerase gamma	1.67
LOC106560721	XM_014123886.1	ankyrin repeat domain-containing protein 26-like	1.67
LOC106584876	XM_014170481.1	uncharacterized LOC106584876	1.67
LOC106587736	XM_014176343.1	mitogen-activated protein kinase 6-like	1.67
LOC106612385	XM_014213478.1	suppressor of cytokine signaling 3-like	1.67
LOC106581932	XM_014164439.1	calcitonin gene-related peptide type 1 receptor	1.67
kmt2a	XM_014213640.1	lysine (K)-specific methyltransferase 2A	1.66
LOC106579141	XM_014158736.1	zinc finger protein 91-like	1.66
LOC106575383	XM_014151888.1	serine/threonine-protein phosphatase 2A regulatory subunit B'' subunit alpha-like	1.66
LOC106565177	XM_014132020.1	voltage-dependent calcium channel subunit alpha-2/delta-2-like	1.66
LOC106593034	XM_014184379.1	STIP1 homology and U box-containing protein 1-like	1.66
LOC106575774	XM_014152454.1	glycerol-3-phosphate dehydrogenase%2C mitochondrial-like	1.66
LOC106561902	XM_014126233.1	protein kinase C-binding protein NELL1-like	1.66
ip6k1	XM_014168331.1	inositol hexakisphosphate kinase 1	1.66
LOC106560308	XM_014123072.1	zinc finger protein 644-like	1.66
LOC106603377	XM_014196969.1	long-chain-fatty-acid--CoA ligase 4-like	1.66
LOC106570630	XM_014143095.1	CLIP-associating protein 2-like	1.66
strn4	XM_014213497.1	striatin%2C calmodulin binding protein 4	1.66
LOC106594520	XM_014185897.1	protein kinase C beta type	1.66
LOC106567057	XM_014135894.1	low-density lipoprotein receptor-related protein 1-like	1.66
LOC106608678	XM_014206777.1	zinc finger and BTB domain-containing protein 38-like	1.65
LOC106565696	XM_014133483.1	rab11 family-interacting protein 5-like	1.65
LOC106581879	XM_014164319.1	type I inositol 3%2C4-bisphosphate 4-phosphatase-like	1.65

LOC106572786	XM_014147257.1	disco-interacting protein 2 homolog B-A-like	1.65
LOC106588578	XM_014177721.1	LIX1-like protein	1.65
LOC106585120	XM_014170969.1	substance-P receptor-like	1.65
LOC106560488	XM_014123453.1	dedicator of cytokinesis protein 7-like	1.65
LOC106602556	XM_014195248.1	protocadherin Fat 1-like	1.65
LOC106564141	XM_014130157.1	protein tweety homolog 3-like	1.65
LOC106595830	XM_014187176.1	LIM and calponin homology domains-containing protein 1-like	1.65
arhgef1	XM_014201601.1	Rho guanine nucleotide exchange factor (GEF) 1	1.65
LOC106608576	XM_014206565.1	ankyrin-2-like	1.65
LOC106609844	XM_014208866.1	exportin-T-like	1.65
LOC106573044	XM_014147667.1	membrane-associated guanylate kinase%2C WW and PDZ domain-containing protein 2-like	1.64
LOC106603506	XM_014197286.1	nesprin-1-like	1.64
LOC106611061	XM_014210912.1	latent-transforming growth factor beta-binding protein 2-like	1.64
LOC106585296	XM_014171366.1	probable E3 ubiquitin-protein ligase HECTD4	1.64
ablim2	XM_014206542.1	actin binding LIM protein family%2C member 2	1.64
LOC106584848	XM_014170451.1	mucin-2-like	1.64
LOC106563331	XM_014128850.1	poly [ADP-ribose] polymerase 14-like	1.64
LOC106607272	XM_014204072.1	F-box/LRR-repeat protein 20	1.64
agt	NM_001140446.1	angiotensinogen (serpin peptidase inhibitor%2C clade A%2C member 8)	1.64
xk	XM_014174210.1	X-linked Kx blood group	1.64
LOC106561234	XM_014124953.1	C-Jun-amino-terminal kinase-interacting protein 2-like	1.64
LOC106586262	XM_014173338.1	dedicator of cytokinesis protein 9-like	1.64
LOC106580084	XM_014160710.1	vacuolar protein sorting-associated protein 13A-like	1.64
LOC106570436	XM_014142770.1	ral guanine nucleotide dissociation stimulator-like	1.64
LOC106584061	XM_014168886.1	terminal uridylyltransferase 4-like	1.64
LOC106568090	XM_014138113.1	amyloid protein-binding protein 2-like	1.63
ubr4	XM_014147487.1	ubiquitin protein ligase E3 component n-recognin 4	1.63
LOC106564512	XM_014130636.1	transcription activator BRG1-like	1.63
LOC106580569	XM_014161766.1	RING finger protein 165-like	1.63
sdccag8	XM_014179967.1	serologically defined colon cancer antigen 8	1.63
map3k12	XM_014134036.1	mitogen-activated protein kinase kinase kinase 12	1.63
trrap	XM_014179710.1	transformation/transcription domain-associated protein	1.63
exd2	XM_014155462.1	exonuclease 3'-5' domain containing 2	1.63

LOC106572005	XM_014145694.1	E3 ubiquitin-protein ligase MIB2-like	1.63
LOC106605615	XM_014201449.1	histone-lysine N-methyltransferase 2A-like	1.63
LOC106582497	XM_014165662.1	integrator complex subunit 6-like	1.63
LOC106599430	XM_014190665.1	laminin subunit gamma-1-like	1.63
LOC106569192	XM_014140291.1	histone-lysine N-methyltransferase 2C-like	1.63
LOC106563328	XM_014128843.1	ankyrin-1-like	1.63
LOC106603889	XM_014198100.1	ubiquitin-like domain-containing CTD phosphatase 1	1.63
LOC106603896	XM_014198112.1	grpE protein homolog 2%2C mitochondrial-like	1.63
LOC106583004	XM_014166712.1	leucine-rich repeats and immunoglobulin-like domains protein 2	1.63
LOC106567782	XM_014137511.1	stAR-related lipid transfer protein 13-like	1.63
LOC106577316	XM_014155272.1	proto-oncogene c-Rel-like	1.63
LOC106565160	XM_014131966.1	serine/threonine-protein kinase WNK2-like	1.62
ppc1a	NM_001146657.1	Phosphatidic acid phosphatase type 2 domain-containing protein 1A	1.62
LOC106587928	XM_014176587.1	C-myc promoter-binding protein-like	1.62
ptchd1	XM_014181864.1	patched domain containing 1	1.62
LOC106576932	XM_014154471.1	heat shock 70 kDa protein 12A-like	1.62
LOC106565828	XM_014133395.1	FYVE%2C RhoGEF and PH domain-containing protein 5-like	1.62
yeats2	XM_014192065.1	YEATS domain containing 2	1.62
LOC106577338	XM_014155305.1	transmembrane protein 145-like	1.62
LOC106609821	XM_014208846.1	putative homeodomain transcription factor 2	1.62
LOC106562605	XM_014127555.1	glutamine and serine-rich protein 1-like	1.62
LOC106572764	XM_014147233.1	ceramide glucosyltransferase-B	1.62
LOC106576490	XM_014153694.1	receptor-type tyrosine-protein phosphatase beta-like	1.62
LOC106588378	XM_014177293.1	major histocompatibility complex class I-related gene protein-like	1.62
LOC106572904	XM_014147488.1	arginine-glutamic acid dipeptide repeats protein-like	1.62
LOC106566301	XM_014134205.1	arginine-glutamic acid dipeptide repeats protein-like	1.62
LOC106582631	XM_014165861.1	receptor-type tyrosine-protein phosphatase T-like	1.62
LOC106603776	XM_014197852.1	ribosomal protein S6 kinase alpha-5-like	1.61
LOC106579490	XM_014159434.1	disco-interacting protein 2 homolog C-like	1.61
LOC106579463	XM_014159394.1	acyl-coenzyme A thioesterase 9%2C mitochondrial-like	1.61
LOC106611247	XM_014211251.1	uncharacterized LOC106611247	1.61
LOC100136526	XM_014131866.1	peroxisome proliferator-activated receptor delta	1.61
LOC106598586	XM_014189618.1	serine/arginine repetitive matrix protein 2-like	1.61

LOC106577155	XM_014154958.1	mitogen-activated protein kinase kinase kinase kinase 3-like	1.61
LOC106578615	XM_014157618.1	receptor-type tyrosine-protein phosphatase N2-like	1.61
LOC106578422	XM_014157181.1	catenin delta-2-like	1.61
LOC106596416	XM_014187709.1	ZZ-type zinc finger-containing protein 3-like	1.61
LOC106613592	XM_014216012.1	dual specificity protein phosphatase CDC14A-like	1.61
LOC106605718	XM_014201621.1	chromodomain-helicase-DNA-binding protein 4-like	1.61
LOC106610732	XM_014210248.1	consortin-like	1.61
LOC106574311	XM_014150144.1	pyridoxal kinase-like	1.61
LOC106567646	XM_014137180.1	opioid-binding protein/cell adhesion molecule-like	1.61
LOC106607167	XM_014203808.1	histone acetyltransferase p300-like	1.61
LOC106575549	XM_014152113.1	uncharacterized LOC106575549	1.61
LOC106563682	XM_014129474.1	H(+)/Cl(-) exchange transporter 5-like	1.61
ptchd2	XM_014160136.1	patched domain containing 2	1.61
LOC106588934	XM_014178493.1	pyruvate dehydrogenase (acetyl-transferring) kinase isozyme 2%2C mitochondrial-like	1.61
LOC106581263	XM_014163213.1	A-kinase anchor protein 1%2C mitochondrial-like	1.60
LOC106572961	XM_014147560.1	ubiquitin-like modifier-activating enzyme 1	1.60
LOC106581600	XM_014163723.1	glioma tumor suppressor candidate region gene 1 protein-like	1.60
aatk	XM_014212696.1	apoptosis-associated tyrosine kinase	1.60
LOC106592637	XM_014183976.1	RNA-binding protein 10-like	1.60
heatr5a	XM_014194302.1	HEAT repeat containing 5A	1.60
LOC106590469	XM_014181530.1	basic helix-loop-helix domain-containing protein KIAA2018-like	1.60
LOC106577519	XM_014155615.1	ras-related protein Rab-1B	1.60
kiaa0226	XM_014149850.1	KIAA0226 ortholog	1.60
LOC106579917	XM_014160293.1	E1A-binding protein p400-like	1.60
LOC106584104	XM_014168941.1	protein furry homolog-like	1.60
LOC106588695	XM_014177955.1	non-canonical poly(A) RNA polymerase PAPD7-like	1.60
LOC106567672	XM_014137229.1	cell surface glycoprotein MUC18-like	1.59
LOC106561041	XM_014124611.1	cadherin EGF LAG seven-pass G-type receptor 1-like	1.59
nbeal1	XM_014149439.1	neurobeachin-like 1	1.59
LOC106567530	XM_014136888.1	protein Jade-1-like	1.59
gpt2	XM_014126510.1	glutamic pyruvate transaminase (alanine aminotransferase) 2	1.59
LOC106602627	XM_014195383.1	spectrin beta chain%2C erythrocytic-like	1.59
LOC106613240	XM_014215308.1	rho guanine nucleotide exchange factor 17-like	1.59

LOC106564302	XM_014130339.1	transportin-2-like	1.59
LOC106589111	XM_014178807.1	gap junction alpha-4 protein-like	1.59
LOC106579616	XM_014159705.1	zinc finger homeobox protein 4-like	1.59
LOC106603108	XM_014196337.1	serine/threonine-protein kinase PAK 3-like	1.59
LOC106592297	XM_014183625.1	G protein-coupled receptor kinase 6-like	1.59
LOC106606681	XM_014203021.1	serine/threonine-protein kinase/endoribonuclease IRE1-like	1.59
LOC106565428	XM_014132551.1	natural resistance-associated macrophage protein 2-like	1.59
LOC106588060	XM_014176726.1	Golgi apparatus protein 1-like	1.59
LOC106571902	XM_014145442.1	IQ motif and SEC7 domain-containing protein 1-like	1.59
LOC106565061	XM_014131688.1	dystroglycan-like	1.58
LOC106565528	XM_014132764.1	beta-1%2C4 N-acetylgalactosaminyltransferase 1-like	1.58
LOC106565476	XM_014132695.1	inositol 1%2C4%2C5-trisphosphate receptor type 1	1.58
LOC106577926	XM_014156395.1	histone-lysine N-methyltransferase 2B-like	1.58
LOC106563220	XM_014128579.1	dihydropyrimidinase-related protein 2	1.58
LOC106571417	XM_014144494.1	transcription factor HIVEP2-like	1.58
LOC106588247	XM_014177065.1	histone-lysine N-methyltransferase 2B-like	1.58
LOC106579406	XM_014159276.1	serine/threonine-protein kinase TAO2-like	1.57
LOC106566442	XM_014134482.1	solute carrier organic anion transporter family member 4A1-like	1.57
LOC106612163	XM_014213099.1	cohesin subunit SA-2-like	1.57
LOC106565096	XM_014131802.1	phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta-like	1.57
LOC106569851	XM_014141529.1	A-kinase anchor protein 9-like	1.57
LOC106593155	XM_014184491.1	serine/arginine repetitive matrix protein 2-like	1.57
LOC106562453	XM_014127345.1	selenocysteine insertion sequence-binding protein 2-like	1.57
LOC106572354	XM_014146414.1	6-phosphofructo-2-kinase/fructose-2%2C6-bisphosphatase 2-like	1.57
LOC106584282	XM_014169349.1	protein zyg-11 homolog	1.57
LOC106597731	XM_014188879.1	serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit A-like	1.57
LOC106600879	XM_014192624.1	synaptogyrin-1-like	1.57
ppp1r9a	XM_014178495.1	protein phosphatase 1%2C regulatory subunit 9A	1.57
LOC106570093	XM_014142078.1	histone-lysine N-methyltransferase ASH1L-like	1.57
LOC106579633	XM_014159734.1	nectin-1-like	1.57
LOC106587237	XM_014175434.1	immunoglobulin superfamily DCC subclass member 4-like	1.57
LOC106583764	XM_014168323.1	arf-GAP with coiled-coil%2C ANK repeat and PH domain-containing protein 3-like	1.57
LOC106577598	XM_014155794.1	excitatory amino acid transporter 3-like	1.56

LOC106609667	XM_014208636.1	alpha-N-acetylneuraminide alpha-2%2C8-sialyltransferase-like	1.56
LOC106574832	XM_014150927.1	R3H domain-containing protein 1-like	1.56
LOC106606314	XM_014202471.1	hepatocyte growth factor-regulated tyrosine kinase substrate-like	1.56
LOC106575618	XM_014152230.1	histone deacetylase 4-like	1.56
LOC106590401	XM_014181380.1	brefeldin A-inhibited guanine nucleotide-exchange protein 1-like	1.56
igsf8	XM_014133993.1	immunoglobulin superfamily%2C member 8	1.56
vps13d	XM_014135237.1	vacuolar protein sorting 13 homolog D (S. cerevisiae)	1.56
LOC106565466	XM_014132678.1	low-density lipoprotein receptor-related protein 1-like	1.56
LOC106569707	XM_014141277.1	zinc finger protein 236-like	1.56
syne1	XM_014205626.1	spectrin repeat containing%2C nuclear envelope 1	1.56
LOC106603258	XM_014196678.1	human immunodeficiency virus type I enhancer-binding protein 2 homolog	1.55
sos2	XM_014193968.1	son of sevenless homolog 2 (Drosophila)	1.55
LOC106584534	XM_014169938.1	Golgi apparatus protein 1-like	1.55
LOC106600538	XM_014191960.1	zinc finger protein 512B-like	1.55
vamp7	XM_014198467.1	vesicle-associated membrane protein 7	1.55
mtmr4	XM_014136579.1	myotubularin related protein 4	1.55
vps13c	XM_014176678.1	vacuolar protein sorting 13 homolog C (S. cerevisiae)	1.55
podxl	XM_014208089.1	podocalyxin-like	1.55
LOC106585950	XM_014172703.1	chondroitin sulfate proteoglycan 4-like	1.55
LOC106594799	XM_014186186.1	zinc finger protein 345-like	1.55
LOC106586162	XM_014173077.1	formin-like protein 2	1.55
LOC106561621	XM_014125750.1	spermatid perinuclear RNA-binding protein-like	1.55
LOC106583663	XM_014168118.1	E3 ubiquitin-protein ligase Itchy-like	1.55
LOC106568365	XM_014138650.1	mucin-5AC-like	1.55
LOC106594751	XM_014186169.1	60 kDa lysophospholipase-like	1.55
LOC106566913	XM_014135516.1	UHRF1-binding protein 1-like	1.54
znf827	XM_014156831.1	zinc finger protein 827	1.54
kifap3	XM_014123520.1	kinesin-associated protein 3	1.54
LOC106601240	XM_014193286.1	protein Tob2-like	1.54
LOC106587065	XM_014174962.1	glycosyltransferase-like protein LARGE2	1.54
atp9b	XM_014177039.1	ATPase%2C class II%2C type 9B	1.54
fndc3b	XM_014150549.1	fibronectin type III domain containing 3B	1.54
LOC106566857	XM_014135352.1	msx2-interacting protein-like	1.54

LOC106575446	XM_014151946.1	cohesin subunit SA-1	1.53
LOC106563084	XM_014128307.1	multiple C2 and transmembrane domain-containing protein 1-like	1.53
hipk3	XM_014125488.1	homeodomain interacting protein kinase 3	1.53
LOC106563569	XM_014129266.1	CD276 antigen homolog	1.53
LOC106610139	XM_014209315.1	BAH and coiled-coil domain-containing protein 1-like	1.53
cables1	XM_014140540.1	Cdk5 and Abl enzyme substrate 1	1.53
tm136	NM_001140640.1	Transmembrane protein 136	1.53
LOC106612277	XM_014213294.1	probable E3 ubiquitin-protein ligase HERC1	1.53
LOC106607536	XM_014204573.1	pleckstrin homology domain-containing family A member 1-like	1.53
LOC106571573	XM_014144796.1	CLOCK-interacting pacemaker-like	1.53
LOC106602727	XM_014195546.1	uncharacterized LOC106602727	1.53
LOC106604536	XM_014199256.1	dihydropteridine reductase-like	1.53
LOC106586971	XM_014174734.1	bromodomain-containing protein 3-like	1.53
LOC106570655	XM_014143124.1	alpha-1%2C3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase-like	1.52
LOC106580341	XM_014161252.1	ankyrin-1-like	1.52
LOC106578639	XM_014157648.1	sickle tail protein-like	1.52
dock11	XM_014206848.1	dedicator of cytokinesis 11	1.52
LOC106611764	XM_014212315.1	cationic amino acid transporter 3-like	1.52
LOC106601392	XM_014193567.1	nucleosome-remodeling factor subunit BPTF-like	1.52
gria4	XM_014215179.1	glutamate receptor%2C ionotropic%2C AMPA 4	1.52
LOC106566343	XM_014134267.1	host cell factor 1-like	1.52
LOC106562918	XM_014128019.1	zinc finger FYVE domain-containing protein 16-like	1.52
ipp	XM_014140732.1	intracisternal A particle-promoted polypeptide	1.52
LOC100380643	XM_014134249.1	filamin-A	1.52
LOC100380701	XM_014191699.1	uncharacterized protein KIAA1109	1.52
LOC106568813	XM_014139483.1	collagen alpha-1(XI) chain-like	1.52
LOC106591672	XM_014182896.1	probable cationic amino acid transporter	1.51
LOC106561920	XM_014126314.1	transcription factor TFIIIB component B'' homolog	1.51
LOC106572349	XM_014146396.1	ankyrin repeat and SAM domain-containing protein 1A-like	1.51
LOC106589879	XM_014180291.1	histone acetyltransferase KAT6B-like	1.51
LOC106576009	XM_014152805.1	multidrug resistance protein 1-like	1.51
LOC106591328	XM_014182539.1	LIM domain-binding protein 1	1.51
akt2	XM_014129610.1	v-akt murine thymoma viral oncogene homolog 2	1.51

LOC106567146	XM_014136107.1	dnaJ homolog subfamily C member 16-like	1.51
gad2	XM_014181019.1	glutamate decarboxylase 2 (pancreatic islets and brain%2C 65kDa)	1.51
LOC106574701	XM_014150712.1	formin-like protein 2	1.51
LOC106576369	XM_014153496.1	UHRF1-binding protein 1-like	1.51
dmxl1	XM_014172539.1	Dmx-like 1	1.50
hecw2	XM_014172970.1	HECT%2C C2 and WW domain containing E3 ubiquitin protein ligase 2	1.50
katnal1	XM_014129722.1	katanin p60 subunit A-like 1	1.50
LOC106569240	XM_014140414.1	calcium/calmodulin-dependent protein kinase type II subunit beta-like	1.50
pxk	XM_014168403.1	PX domain containing serine/threonine kinase	1.50
LOC106613957	XM_014216815.1	ras-specific guanine nucleotide-releasing factor RalGPS2	1.50
LOC106588368	XM_014177277.1	zinc fingers and homeoboxes protein 2-like	1.50
LOC106593684	XM_014185056.1	uncharacterized LOC106593684	1.50
LOC106565749	XM_014133211.1	inositol 1%2C4%2C5-trisphosphate receptor type 1-like	1.50
LOC106579810	XM_014160007.1	volume-regulated anion channel subunit LRRC8A-like	1.50
LOC106578854	XM_014158078.1	growth arrest-specific protein 7-like	1.50
LOC106611718	XM_014212219.1	sodium/hydrogen exchanger 6-like	1.50
LOC106572956	XM_014147548.1	uncharacterized LOC106572956	1.50
parp16	XM_014170240.1	poly (ADP-ribose) polymerase family%2C member 16	1.50
acaca	XM_014138098.1	acetyl-CoA carboxylase alpha	1.49
LOC106584996	XM_014170713.1	putative phospholipase B-like 2	1.49
ptpn9	XM_014124056.1	protein tyrosine phosphatase%2C non-receptor type 9	1.49
dennd5a	XM_014124661.1	DENN/MADD domain containing 5A	1.49
LOC106569807	XM_014141433.1	serine/threonine-protein kinase OSR1-like	1.49
LOC106564026	XM_014129998.1	zinc finger protein 239-like	1.49
sfxn1	XM_014213238.1	sideroflexin 1	1.49
lama2	XM_014205066.1	laminin%2C alpha 2	1.49
LOC106602006	XM_014194447.1	ubiquitin carboxyl-terminal hydrolase 7-like	1.49
LOC106564108	XM_014130101.1	bifunctional apoptosis regulator-like	1.49
LOC106607385	XM_014204301.1	KN motif and ankyrin repeat domain-containing protein 2-like	1.49
LOC106583011	XM_014166738.1	ankyrin repeat and SAM domain-containing protein 1A-like	1.49
LOC106586633	XM_014174126.1	MORC family CW-type zinc finger protein 3-like	1.49
LOC106607262	XM_014204055.1	synaptic vesicle membrane protein VAT-1 homolog	1.49
LOC106583141	XM_014166998.1	low-density lipoprotein receptor-related protein 1-like	1.49

LOC100286503	XM_014164404.1	tensin-1	1.48
LOC106583613	XM_014168006.1	plectin-like	1.48
rab12	XM_014141315.1	RAB12%2C member RAS oncogene family	1.48
LOC106585250	XM_014171261.1	oxysterol-binding protein 2-like	1.48
LOC106612325	XM_014213377.1	transcriptional regulator ATRX-like	1.48
LOC106574130	XM_014149749.1	arf-GAP with coiled-coil%2C ANK repeat and PH domain-containing protein 2-like	1.48
tmtc3	XM_014125664.1	transmembrane and tetratricopeptide repeat containing 3	1.48
cfap54	XM_014195735.1	cilia and flagella associated 54	1.48
LOC106583229	XM_014167156.1	signal transducer and activator of transcription 1-like	1.48
LOC106585077	XM_014170855.1	tankyrase-1	1.48
LOC106609299	XM_014207957.1	filamin-C-like	1.48
LOC106560551	XM_014123546.1	protein crumbs homolog 1-like	1.48
LOC106601875	XM_014194301.1	ubiquitin carboxyl-terminal hydrolase 22-like	1.48
LOC106563684	XM_014129482.1	ras-related protein Rab-39B-like	1.48
LOC106562084	XM_014126644.1	nuclear mitotic apparatus protein 1-like	1.47
LOC106586635	XM_014174131.1	uncharacterized LOC106586635	1.47
LOC106608762	XM_014206884.1	chromodomain-helicase-DNA-binding protein 3-like	1.47
LOC106570670	XM_014143139.1	phosphatidylinositide phosphatase SAC1-B	1.47
LOC106570016	XM_014141877.1	microtubule-actin cross-linking factor 1-like	1.47
LOC106563702	XM_014129516.1	RNA-binding protein 4-like	1.47
LOC106612640	XM_014214040.1	ribosomal protein S6 kinase alpha-1	1.47
LOC106592516	XM_014183860.1	uncharacterized LOC106592516	1.47
LOC106583546	XM_014167850.1	uncharacterized LOC106583546	1.47
LOC106576946	XM_014154494.1	baculoviral IAP repeat-containing protein 6-like	1.47
akap11	XM_014173904.1	A kinase (PRKA) anchor protein 11	1.47
LOC106573267	XM_014148176.1	zinc finger CCCH domain-containing protein 13-like	1.47
kndc1	XM_014200072.1	kinase non-catalytic C-lobe domain (KIND) containing 1	1.47
LOC106601226	XM_014193262.1	transmembrane protein 184B-like	1.47
LOC106581996	XM_014164618.1	type I inositol 3%2C4-bisphosphate 4-phosphatase-like	1.47
LOC106602602	XM_014195339.1	ATP-binding cassette sub-family A member 1-like	1.46
LOC106603277	XM_014196713.1	seizure protein 6-like	1.46
usp24	XM_014169242.1	ubiquitin specific peptidase 24	1.46
LOC106583648	XM_014168079.1	potassium voltage-gated channel subfamily KQT member 2-like	1.46

LOC106567418	XM_014136630.1	teneurin-2-like	1.46
LOC106593144	XM_014184482.1	zinc finger protein 883-like	1.46
mllt4	XM_014205151.1	myeloid/lymphoid or mixed-lineage leukemia%3B translocated to%2C 4	1.46
gtf3c3	XM_014185889.1	general transcription factor IIIC%2C polypeptide 3%2C 102kDa	1.46
LOC106580770	XM_014162162.1	uncharacterized LOC106580770	1.46
LOC106603436	XM_014197114.1	NADPH--cytochrome P450 reductase-like	1.46
LOC106603839	XM_014198021.1	janus kinase and microtubule-interacting protein 2-like	1.46
LOC106593919	XM_014185295.1	WD repeat domain phosphoinositide-interacting protein 2-like	1.46
LOC106608076	XM_014205763.1	MAX gene-associated protein-like	1.45
LOC106568720	XM_014139300.1	tyrosine-protein kinase JAK1-like	1.45
LOC106584072	XM_014168912.1	desmoplakin-like	1.45
LOC106608363	XM_014206275.1	tau-tubulin kinase 1-like	1.45
hgsnat	XM_014181930.1	heparan-alpha-glucosaminide N-acetyltransferase	1.45
LOC106576347	XM_014153470.1	glutamate receptor ionotropic%2C kainate 5-like	1.45
trip11	XM_014211716.1	thyroid hormone receptor interactor 11	1.45
LOC106580491	XM_014161618.1	histone acetyltransferase KAT6A-like	1.45
LOC106578133	XM_014156747.1	leucine-rich repeat and fibronectin type-III domain-containing protein 2-like	1.45
LOC106569517	XM_014140950.1	semaphorin-6B-like	1.45
LOC106610679	XM_014210140.1	nuclear receptor coactivator 7-like	1.44
LOC106604312	XM_014198827.1	ankyrin repeat and KH domain-containing protein 1-like	1.44
LOC106590181	XM_014180923.1	gamma-adducin-like	1.44
LOC106568339	XM_014138599.1	tyrosine-protein kinase Fer-like	1.44
LOC106582182	XM_014164995.1	E3 SUMO-protein ligase RanBP2-like	1.44
LOC106560827	XM_014124168.1	ran-binding protein 10-like	1.44
ralbp1	XM_014141319.1	ralA binding protein 1	1.44
LOC106563875	XM_014129799.1	protocadherin-16-like	1.44
LOC106600808	XM_014192497.1	zinc finger protein 91-like	1.44
LOC100380858	XM_014172839.1	E3 ubiquitin-protein ligase MYCBP2	1.44
LOC106582213	XM_014165061.1	E3 SUMO-protein ligase RanBP2-like	1.44
LOC106575498	XM_014152012.1	probable ubiquitin carboxyl-terminal hydrolase FAF-X	1.44
LOC106612937	XM_014214612.1	multidrug and toxin extrusion protein 1-like	1.44
LOC106608394	XM_014206306.1	zinc finger MIZ domain-containing protein 1-like	1.44
bahcc1	XM_014179743.1	BAH domain and coiled-coil containing 1	1.44

LOC106612256	XM_014213255.1	proton-coupled amino acid transporter 1-like	1.44
LOC106608676	XM_014206776.1	DNA-directed RNA polymerase II subunit RPB1-like	1.44
LOC106562962	XM_014128097.1	collagen alpha-1(V) chain-like	1.44
LOC106588182	XM_014176914.1	histone-lysine N-methyltransferase ASH1L-like	1.44
LOC106585522	XM_014171824.1	E1A-binding protein p400-like	1.43
daam1	XM_014211140.1	dishevelled associated activator of morphogenesis 1	1.43
LOC106605802	XM_014201748.1	regulation of nuclear pre-mRNA domain-containing protein 2-like	1.43
LOC106566627	XM_014134877.1	arf-GAP with SH3 domain%2C ANK repeat and PH domain-containing protein 2-like	1.43
LOC106590504	XM_014181589.1	thyroid hormone receptor-associated protein 3-like	1.43
LOC106599001	XM_014190038.1	microtubule-associated serine/threonine-protein kinase 3-like	1.43
LOC106593948	XM_014185325.1	uncharacterized LOC106593948	1.43
LOC106563729	XM_014129555.1	amyloid beta A4 precursor protein-binding family B member 3-like	1.43
LOC106604257	XM_014198738.1	rho-related GTP-binding protein RhoG-like	1.43
gpr18	XM_014163774.1	G protein-coupled receptor 18	1.43
LOC106576468	XM_014153670.1	adiponectin receptor protein 2-like	1.43
LOC106589933	XM_014180385.1	calcineurin subunit B type 1-like	1.42
LOC106610929	XM_014210609.1	gamma-aminobutyric acid receptor subunit beta-1-like	1.42
LOC106613135	XM_014215095.1	ephrin type-A receptor 4-like	1.42
LOC106603151	XM_014196451.1	intersectin-2-like	1.42
LOC106577113	XM_014154855.1	echinoderm microtubule-associated protein-like 6	1.42
LOC106597009	XM_014188247.1	endophilin-A1-like	1.42
LOC106562151	XM_014126810.1	tight junction protein ZO-1-like	1.42
LOC106613723	XM_014216273.1	protein strawberry notch homolog 2-like	1.42
LOC106584705	XM_014170219.1	transcription factor PU.1-like	1.42
LOC106584317	XM_014169431.1	centrosome-associated protein 350-like	1.42
LOC106606244	XM_014202392.1	ubiquitin carboxyl-terminal hydrolase 7-like	1.42
orai2	XM_014197169.1	ORAI calcium release-activated calcium modulator 2	1.42
LOC106584620	XM_014170105.1	transcription factor SOX-6-like	1.42
LOC106580886	XM_014162419.1	uncharacterized LOC106580886	1.42
LOC106607739	XM_014205013.1	DNA polymerase zeta catalytic subunit-like	1.42
LOC106583293	XM_014167280.1	gastrula zinc finger protein XlCGF58.1-like	1.42
LOC106586757	XM_014174358.1	serine/threonine-protein kinase 10-like	1.42
LOC106562326	XM_014127130.1	synaptotagmin-7-like	1.42

LOC106589530	XM_014179626.1	CREB-binding protein-like	1.42
LOC106565100	XM_014131804.1	pleckstrin homology domain-containing family A member 6-like	1.42
LOC100380641	XM_014147119.1	E3 ubiquitin-protein ligase HUWE1	1.42
LOC106568668	XM_014139191.1	FAST kinase domain-containing protein 3-like	1.41
LOC106606113	XM_014202234.1	SNF-related serine/threonine-protein kinase-like	1.41
LOC106560231	XM_014122872.1	ubiquitin carboxyl-terminal hydrolase 24-like	1.41
LOC106600551	XM_014191988.1	serine/threonine-protein kinase OSR1-like	1.41
kbtbd4	XM_014126108.1	kelch repeat and BTB (POZ) domain containing 4	1.41
LOC106566213	XM_014134088.1	protein VPRBP-like	1.41
LOC106585556	XM_014171906.1	dihydropyrimidinase-related protein 2-like	1.41
LOC106587652	XM_014176230.1	paired amphipathic helix protein Sin3a-like	1.41
LOC106588461	XM_014177495.1	microtubule-actin cross-linking factor 1-like	1.41
LOC106595592	XM_014186959.1	SCY1-like protein 2	1.41
LOC106565273	XM_014132192.1	ral GTPase-activating protein subunit beta-like	1.41
LOC106586144	XM_014173048.1	RNA-binding protein 34-like	1.40
herc2	XM_014215599.1	HECT and RLD domain containing E3 ubiquitin protein ligase 2	1.40
ncor1	XM_014137920.1	nuclear receptor corepressor 1	1.40
ankrd12	XM_014142934.1	ankyrin repeat domain 12	1.40
LOC106560257	XM_014122934.1	protein furry homolog-like	1.40
LOC106604099	XM_014198491.1	transcriptional regulator ATRX-like	1.40
LOC106607615	XM_014204761.1	protein FAM149B1-like	1.40
LOC106603121	XM_014196383.1	sodium/potassium/calcium exchanger 3-like	1.40
LOC106566780	XM_014135168.1	cadherin EGF LAG seven-pass G-type receptor 3-like	1.40
LOC106576747	XM_014154115.1	echinoderm microtubule-associated protein-like 4	1.40
LOC106566398	XM_014134378.1	zinc finger protein 335-like	1.40
LOC106571127	XM_014143830.1	kinesin-like protein KIF26A	1.40
LOC106567026	XM_014135807.1	E3 ubiquitin-protein ligase HUWE1-like	1.40
LOC106606345	XM_014202500.1	protein NLRC3-like	1.40
LOC106613785	XM_014216397.1	single-stranded DNA-binding protein 3-like	1.40
pi4ka	XM_014127721.1	phosphatidylinositol 4-kinase%2C catalytic%2C alpha	1.39
atl1	XM_014194332.1	atlastin GTPase 1	1.39
LOC106607387	XM_014204302.1	dedicator of cytokinesis protein 7-like	1.39
LOC106581247	XM_014163167.1	unconventional myosin-XVIIIa-like	1.39

LOC106571938	XM_014145520.1	bromodomain adjacent to zinc finger domain protein 2A-like	1.39
LOC106586745	XM_014174319.1	DDB1- and CUL4-associated factor 6-like	1.39
celsr1	XM_014170066.1	cadherin%2C EGF LAG seven-pass G-type receptor 1	1.39
LOC106574232	XM_014149951.1	lymphocyte antigen 75-like	1.39
mdn1	XM_014204960.1	midasin AAA ATPase 1	1.39
LOC106585779	XM_014172394.1	ubiquitin carboxyl-terminal hydrolase 34-like	1.39
LOC106579993	XM_014160495.1	voltage-dependent N-type calcium channel subunit alpha-1B-like	1.39
LOC106574324	XM_014150166.1	ATP-binding cassette sub-family B member 6%2C mitochondrial-like	1.39
LOC106586434	XM_014173706.1	low-density lipoprotein receptor-related protein 1B-like	1.39
LOC106569317	XM_014140546.1	E3 ubiquitin-protein ligase mib1-like	1.39
LOC106604853	XM_014199910.1	carnosine synthase 1-like	1.39
LOC106608924	XM_014207147.1	histone acetyltransferase p300-like	1.39
LOC106601059	XM_014192961.1	dedicator of cytokinesis protein 7-like	1.39
LOC106607502	XM_014204506.1	zinc finger protein 646-like	1.39
LOC106570121	XM_014142151.1	histone-lysine N-methyltransferase 2B-like	1.39
LOC106613609	XM_014216043.1	microtubule-associated protein 1B-like	1.39
gpr19	XM_014183397.1	G protein-coupled receptor 19	1.39
LOC106608644	XM_014206720.1	DENN domain-containing protein 4C-like	1.39
arhgap31	XM_014129333.1	Rho GTPase activating protein 31	1.39
arvcf	XM_014170897.1	armadillo repeat gene deleted in velocardiofacial syndrome	1.39
apc	XM_014126219.1	adenomatous polyposis coli	1.39
LOC106572865	XM_014147408.1	sortilin-like	1.39
LOC106582376	XM_014165433.1	isocitrate dehydrogenase [NADP] cytoplasmic-like	1.39
LOC106583532	XM_014167833.1	potassium voltage-gated channel subfamily B member 1-like	1.38
LOC106580939	XM_014162499.1	pleckstrin homology-like domain family B member 1	1.38
LOC106588595	XM_014177750.1	poly [ADP-ribose] polymerase 10-like	1.38
LOC106572710	XM_014147116.1	histone lysine demethylase PHF8-like	1.38
bicc1	XM_014154784.1	BicC family RNA binding protein 1	1.38
LOC106610958	XM_014210685.1	BAG family molecular chaperone regulator 5-like	1.38
baz2b	XM_014164143.1	bromodomain adjacent to zinc finger domain%2C 2B	1.38
LOC106577192	XM_014155046.1	disks large homolog 5-like	1.38
LOC106561374	XM_014125257.1	zinc finger protein 462-like	1.37
rb39b	NM_001140706.1	Ras-related protein Rab-39B	1.37

camkk2	XM_014134123.1	calcium/calmodulin-dependent protein kinase kinase 2%2C beta	1.37
LOC106611354	XM_014211507.1	transcription factor HIVEP2-like	1.37
LOC106571901	XM_014145440.1	nuclear pore membrane glycoprotein 210-like	1.37
LOC106606894	XM_014203350.1	uncharacterized LOC106606894	1.37
LOC106609167	XM_014207647.1	uncharacterized LOC106609167	1.37
LOC106607945	XM_014205458.1	attractin-like protein 1	1.37
LOC106609880	XM_014208904.1	ATP-binding cassette sub-family A member 1-like	1.37
LOC106597319	XM_014188527.1	serine/threonine-protein kinase PRP4 homolog	1.37
LOC106612372	XM_014213449.1	rho GTPase-activating protein 35-like	1.37
LOC106568905	XM_014139698.1	glycogen debranching enzyme-like	1.37
LOC106613304	XM_014215396.1	disks large homolog 5-like	1.36
LOC106587244	XM_014175447.1	tight junction protein ZO-1-like	1.36
celsr3	XM_014146319.1	cadherin%2C EGF LAG seven-pass G-type receptor 3	1.36
LOC106608277	XM_014206138.1	stathmin-4-like	1.36
LOC106566935	XM_014135550.1	mediator of RNA polymerase II transcription subunit 13-like	1.36
LOC106572922	XM_014147508.1	host cell factor 1-like	1.36
setd1b	XM_014130654.1	SET domain containing 1B	1.36
LOC106569615	XM_014141157.1	hippocampus abundant transcript 1 protein-like	1.36
LOC106566730	XM_014135058.1	death-inducer obliterator 1-like	1.36
fgfr2	XM_014154423.1	fibroblast growth factor receptor 2	1.36
LOC106581949	XM_014164507.1	probable ubiquitin carboxyl-terminal hydrolase FAF-X	1.36
LOC106564780	XM_014131144.1	proteoglycan 4-like	1.36
LOC106603505	XM_014197279.1	nuclear receptor corepressor 1-like	1.35
LOC106573031	XM_014147639.1	activating molecule in BECN1-regulated autophagy protein 1-like	1.35
myo6	XM_014204923.1	myosin VI	1.35
LOC106567420	XM_014136642.1	ankyrin repeat and KH domain-containing protein 1-like	1.35
LOC106574260	XM_014150023.1	CLIP-associating protein 1-B-like	1.35
LOC106606839	XM_014203218.1	cAMP-dependent protein kinase catalytic subunit alpha	1.34
LOC106606438	XM_014202644.1	E3 ubiquitin-protein ligase RNF213-like	1.34
mapkapk5	XM_014160995.1	mitogen-activated protein kinase-activated protein kinase 5	1.34
LOC106609404	XM_014208197.1	golgin subfamily B member 1-like	1.34
LOC106606187	XM_014202318.1	putative oxidoreductase GLYR1	1.34
LOC106609364	XM_014208124.1	myotubularin-related protein 5-like	1.34

LOC106560961	XM_014124440.1	A-kinase anchor protein 13-like	1.34
LOC106586143	XM_014173040.1	glutaminase kidney isoform%2C mitochondrial-like	1.34
LOC106587346	XM_014175667.1	transcription factor 12-like	1.33
LOC106570019	XM_014141900.1	zinc finger MYM-type protein 4-like	1.33
LOC106601535	XM_014193789.1	histone-lysine N-methyltransferase SETD1A-like	1.33
cssa01h5orf42	XM_014130125.1	chromosome ssa01 open reading frame%2C human C5orf42	1.33
akap12	XM_014205622.1	A kinase (PRKA) anchor protein 12	1.33
LOC106604460	XM_014199094.1	mediator of RNA polymerase II transcription subunit 12-like	1.33
LOC106610401	XM_014209766.1	putative sodium-coupled neutral amino acid transporter 10	1.33
LOC106588825	XM_014178285.1	zinc finger protein OZF-like	1.33
LOC106603093	XM_014196293.1	nuclear factor related to kappa-B-binding protein-like	1.33
LOC106584099	XM_014168932.1	uncharacterized LOC106584099	1.33
LOC106594832	XM_014186218.1	mitogen-activated protein kinase 6-like	1.33
tenm3	XM_014199200.1	teneurin transmembrane protein 3	1.33
LOC106577869	XM_014156319.1	ral GTPase-activating protein subunit alpha-2-like	1.33
LOC106573797	XM_014149146.1	uncharacterized LOC106573797	1.32
LOC106582878	XM_014166456.1	host cell factor 1-like	1.32
LOC106590111	XM_014180690.1	disks large-associated protein 1-like	1.32
LOC106599852	XM_014191197.1	ATP-binding cassette sub-family B member 8%2C mitochondrial-like	1.32
LOC106591038	XM_014182227.1	transcriptional regulator ATRX-like	1.32
LOC106594851	XM_014186236.1	vacuolar protein-sorting-associated protein 36-like	1.32
LOC106608443	XM_014206375.1	zinc finger protein 638-like	1.32
LOC106577937	XM_014156410.1	solute carrier family 12 member 6-like	1.32
tenm2	XM_014198165.1	teneurin transmembrane protein 2	1.32
kiaa1033	XM_014169894.1	KIAA1033 ortholog	1.32
LOC106609564	XM_014208501.1	UHRF1-binding protein 1-like	1.32
LOC106578687	XM_014157789.1	titin-like	1.32
LOC106565871	XM_014133495.1	leucine-rich repeats and immunoglobulin-like domains protein 2	1.32
LOC106579475	XM_014159419.1	uncharacterized protein CXorf23-like	1.32
LOC106563799	XM_014129659.1	telomerase-binding protein EST1A-like	1.31
LOC106577550	XM_014155668.1	DNA-directed RNA polymerase II subunit RPB1	1.31
dync1h1	XM_014211261.1	dynein%2C cytoplasmic 1%2C heavy chain 1	1.31
LOC106575550	XM_014152116.1	ATP-dependent RNA helicase DDX3X-like	1.31

LOC106572432	XM_014146609.1	lysine-specific demethylase 5C-like	1.31
LOC106585679	XM_014172162.1	probable helicase with zinc finger domain	1.31
LOC106563017	XM_014128196.1	microtubule-associated serine/threonine-protein kinase 1-like	1.31
zfhx2	XM_014181083.1	zinc finger homeobox 2	1.31
LOC106578096	XM_014156690.1	uncharacterized LOC106578096	1.31
LOC106569888	XM_014141603.1	uncharacterized LOC106569888	1.30
LOC106603594	XM_014197462.1	zinc finger protein 287-like	1.30
LOC106584163	XM_014169101.1	uncharacterized protein KIAA1107-like	1.30
atg2b	XM_014182999.1	autophagy related 2B	1.30
LOC106570138	XM_014142175.1	protein capicua homolog	1.30
LOC106567000	XM_014135743.1	disco-interacting protein 2 homolog B-A	1.29
LOC106583438	XM_014167597.1	helicase ARIP4-like	1.29
cep152	XM_014126006.1	centrosomal protein 152kDa	1.29
LOC106563574	XM_014129280.1	APC membrane recruitment protein 2-like	1.29
LOC106579416	XM_014159306.1	glutamine and serine-rich protein 1-like	1.29
LOC106563499	XM_014129150.1	leucine-rich repeat and fibronectin type-III domain-containing protein 4-like	1.29
LOC106605930	XM_014201937.1	cullin-9-like	1.29
tsc1	XM_014174820.1	tuberous sclerosis 1	1.29
LOC106580217	XM_014161045.1	zinc finger CCCH domain-containing protein 13-like	1.29
prkar1a	XM_014179783.1	protein kinase%2C cAMP-dependent%2C regulatory%2C type I%2C alpha	1.29
LOC106580171	XM_014160933.1	glutamate receptor ionotropic%2C delta-2-like	1.28
LOC106605771	XM_014201700.1	uncharacterized LOC106605771	1.28
LOC106608118	XM_014205839.1	serine/threonine-protein kinase MRCK alpha-like	1.28
LOC106603445	XM_014197130.1	E3 ubiquitin-protein ligase TTC3-like	1.28
LOC106564572	XM_014130716.1	choline transporter-like protein 2	1.28
LOC106603740	XM_014197790.1	rho guanine nucleotide exchange factor 12-like	1.27
LOC100380626	XM_014211175.1	E3 ubiquitin-protein ligase HECTD1	1.27
LOC106563530	XM_014129206.1	anoctamin-5-like	1.27
LOC106584401	XM_014169676.1	AP-3 complex subunit delta-1-like	1.26
LOC106567856	XM_014137676.1	transcription initiation factor TFIID subunit 1-like	1.25
LOC106607579	XM_014204654.1	ELM2 and SANT domain-containing protein 1-like	1.24
LOC106561878	XM_014126152.1	bifunctional glutamate/proline--tRNA ligase-like	1.24
arhgap5	XM_014194348.1	Rho GTPase activating protein 5	1.24

LOC106566086	XM_014133916.1	protein FAM208A-like	1.23
LOC106590587	XM_014181700.1	E3 ubiquitin-protein ligase MARCH6-like	1.23
LOC106563887	XM_014129821.1	nipped-B-like protein	1.22
LOC106605925	XM_014201929.1	uncharacterized LOC106605925	1.21

Table S2. Downregulated (false discovery rate (FDR) < 0.01) genes in Atlantic salmon parr subjected to 8 weeks of volitional exercise, compared to unexercised controls, expressed as fold change (FC) differences.

Gene name	Transcript ID	Product	FC
LOC106576770	XM_014154148.1	placenta-specific protein 9-like	2.24
LOC106567285	XM_014136415.1	protein phosphatase 1 regulatory subunit 12B-like	2.17
LOC106597403	XM_014188595.1	60S ribosomal protein L30-like	2.06
LOC106567650	XM_014137192.1	interleukin-31 receptor subunit alpha-like	2.06
LOC106605470	XM_014201175.1	calbindin-like	2.05
ier2	NM_001140121.1	immediate early response 2	2.04
ccd93	NM_001140793.1	Coiled-coil domain-containing protein 93	2.01
gmnn	XM_014178281.1	geminin%2C DNA replication inhibitor	2.01
LOC106600784	XM_014192436.1	uncharacterized LOC106600784	2.01
LOC106607261	XM_014204054.1	uncharacterized LOC106607261	2.00
LOC106595025	XM_014186406.1	uncharacterized LOC106595025	1.97
foxn4	XM_014160780.1	forkhead box N4	1.94
LOC106586018	XM_014172799.1	P2Y purinoceptor 8-like	1.87
mrps25	XM_014134839.1	mitochondrial ribosomal protein S25	1.82
tprkb	NM_001146530.1	TP53RK binding protein	1.79
LOC106560857	XM_014124239.1	testican-2-like	1.77
LOC106601135	XM_014193096.1	formin-like protein 1	1.77
LOC106573097	XM_014147799.1	sorting nexin-10B-like	1.76
arhgap22	XM_014198771.1	Rho GTPase activating protein 22	1.74
LOC106577931	XM_014156403.1	gamma-secretase subunit PEN-2	1.74
LOC106610322	XM_014209609.1	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6-like	1.74
LOC106566918	XM_014135522.1	SAM pointed domain-containing Ets transcription factor-like	1.73
LOC106595643	XM_014187007.1	uncharacterized protein C11orf98 homolog	1.72
LOC106609350	XM_014208095.1	PHD finger-like domain-containing protein 5A	1.71
anc13	NM_001146659.1	Anaphase-promoting complex subunit 13	1.71
LOC106610173	XM_014209364.1	polypeptide N-acetylgalactosaminyltransferase-like 6	1.71
LOC106570689	XM_014143162.1	MARCKS-related protein-like	1.70
LOC106572841	XM_014147371.1	small nuclear ribonucleoprotein G	1.70
LOC106579659	XM_014159793.1	GTP-binding protein Rheb	1.70

LOC106581057	XM_014162767.1	coiled-coil-helix-coiled-coil-helix domain-containing protein 2-like	1.69
LOC106566856	XM_014135351.1	succinate dehydrogenase [ubiquinone] iron-sulfur subunit%2C mitochondrial-like	1.69
LOC106598275	XM_014189325.1	G patch domain and KOW motifs-containing protein-like	1.69
LOC106605408	XM_014201013.1	zinc-binding protein A33-like	1.68
LOC106609479	XM_014208352.1	small nuclear ribonucleoprotein F	1.68
LOC106567806	XM_014137561.1	non-histone chromosomal protein HMG-14A-like	1.68
LOC106607185	XM_014203875.1	von Willebrand factor C domain-containing protein 2-like	1.68
LOC106601291	XM_014193395.1	growth arrest and DNA damage-inducible proteins-interacting protein 1-like	1.67
LOC106604695	XM_014199616.1	short coiled-coil protein B-like	1.67
LOC106566187	XM_014134060.1	translation machinery-associated protein 7	1.66
LOC106591659	XM_014182884.1	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7-like	1.66
LOC106602949	XM_014195997.1	protein CXorf40A-like	1.66
LOC106609122	XM_014207571.1	vesicle transport protein GOT1B-like	1.65
LOC106565911	XM_014133598.1	mRNA turnover protein 4 homolog	1.65
LOC106593061	XM_014184408.1	meteorin-like protein	1.65
LOC106601365	XM_014193542.1	transcription elongation factor 1 homolog	1.64
LOC106600265	XM_014191583.1	NEDD8-like	1.64
ndub7	NM_001140935.1	NADH dehydrogenase 1 beta subcomplex subunit 7	1.64
LOC106583717	XM_014168243.1	translation machinery-associated protein 7-like	1.63
LOC106565650	XM_014132981.1	MICOS complex subunit MIC10-like	1.63
LOC106566032	XM_014133838.1	60S acidic ribosomal protein P2-like	1.63
LOC106560581	XM_014123596.1	prothymosin alpha-A-like	1.63
LOC106561290	XM_014125077.1	uncharacterized LOC106561290	1.63
LOC106565357	XM_014132369.1	mitochondrial import receptor subunit TOM6 homolog	1.63
LOC106605614	XM_014201448.1	gamma-secretase subunit PEN-2-like	1.62
LOC106573476	XM_014148563.1	proteoglycan 4-like	1.62
LOC106581904	XM_014164352.1	NADH dehydrogenase [ubiquinone] flavoprotein 3%2C mitochondrial-like	1.62
LOC106570471	XM_014142851.1	26S proteasome complex subunit DSS1-like	1.62
LOC106568858	XM_014139592.1	integral membrane protein 2C-like	1.61
rl23a	NM_001141301.1	60S ribosomal protein L23a	1.61
LOC106606605	XM_014202931.1	hematological and neurological expressed 1 protein-like	1.61
LOC106608853	XM_014207032.1	homeobox and leucine zipper protein Homez-like	1.61
lsm5	NM_001146660.1	U6 snRNA-associated Sm-like protein LSm5	1.61

mak16	XM_014174880.1	MAK16 homolog	1.61
LOC106601801	XM_014194188.1	hematological and neurological expressed 1 protein-like	1.60
LOC106576397	XM_014153539.1	39S ribosomal protein L42%2C mitochondrial-like	1.60
srek1ip1	XM_014126946.1	SREK1-interacting protein 1	1.60
LOC106562913	XM_014128007.1	histone H2A.V	1.60
LOC106586742	XM_014174318.1	cytochrome c oxidase assembly factor 5	1.59
LOC106582218	XM_014165073.1	stress-associated endoplasmic reticulum protein 2	1.59
LOC106585031	XM_014170775.1	28S ribosomal protein S36%2C mitochondrial-like	1.59
LOC106561682	XM_014125865.1	small nuclear ribonucleoprotein F-like	1.59
LOC106611745	XM_014212286.1	cysteine-rich hydrophobic domain-containing protein 1-like	1.59
ppdpf	NM_001139699.1	c20orf149 protein	1.58
cxxc5	XM_014212997.1	CXXC finger protein 5	1.58
ca108	NM_001140609.2	CA108 protein	1.58
LOC106600555	XM_014192003.1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13-like	1.58
LOC106561079	XM_014124701.1	40S ribosomal protein S13	1.58
LOC106598980	XM_014190015.1	40S ribosomal protein S28	1.58
LOC106584327	XM_014169486.1	stathmin-like	1.58
LOC106611775	XM_014212334.1	short coiled-coil protein B-like	1.57
dnajc12	NM_001141838.1	DnaJ (Hsp40) homolog%2C subfamily C%2C member 12	1.57
hn1	NM_001123720.1	hematological and neurological expressed 1	1.57
LOC106581844	XM_014164255.1	RNA-binding protein 8A	1.56
cssa15h14orf2	XM_014144682.1	chromosome ssa15 open reading frame%2C human C14orf2	1.56
dynlt3	XM_014174208.1	dynein%2C light chain%2C Tctex-type 3	1.56
LOC106583768	XM_014168332.1	transcription and mRNA export factor ENY2-1	1.56
LOC106611096	XM_014210963.1	proto-oncogene c-Fos-like	1.56
LOC106588702	XM_014177965.1	NADH dehydrogenase [ubiquinone] iron-sulfur protein 6%2C mitochondrial-like	1.56
ndub6	NM_001140996.2	NADH dehydrogenase 1 beta subcomplex subunit 6	1.55
LOC106609885	XM_014208916.1	complexin-1-like	1.55
hint1	NM_001141156.1	histidine triad nucleotide binding protein 1	1.55
LOC106573685	XM_014149000.1	cytochrome c oxidase subunit 7A2%2C mitochondrial-like	1.55
LOC106583512	XM_014167784.1	mitotic-spindle organizing protein 1	1.55
LOC106599020	XM_014190066.1	transcription factor jun-D-like	1.55
lsm3	NM_001146369.1	LSM3 homolog%2C U6 small nuclear RNA and mRNA degradation associated	1.55

atp5e	XM_014145574.1	ATP synthase%2C H+ transporting%2C mitochondrial F1 complex%2C epsilon subunit	1.55
LOC106594622	XM_014186029.1	ubiquitin-like protein 5	1.55
ct011	NM_001140653.1	CT011 protein	1.55
LOC106579627	XM_014159725.1	programmed cell death protein 6-like	1.55
LOC106596057	XM_014187377.1	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8%2C mitochondrial-like	1.55
ofd1	XM_014164391.1	oral-facial-digital syndrome 1	1.55
LOC106571604	XM_014144864.1	splicing factor 3B subunit 6	1.55
LOC106583016	XM_014166754.1	signal peptidase complex subunit 1-like	1.54
LOC106607575	XM_014204646.1	zinc finger CCHC domain-containing protein 10-like	1.54
LOC106611374	XM_014211536.1	SRA stem-loop-interacting RNA-binding protein%2C mitochondrial-like	1.54
LOC106577308	XM_014155256.1	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8%2C mitochondrial-like	1.53
fundc2	XM_014129171.1	FUN14 domain containing 2	1.53
LOC106604773	XM_014199794.1	39S ribosomal protein L18%2C mitochondrial-like	1.53
LOC106572526	XM_014146767.1	zinc transporter ZIP10-like	1.53
LOC106604775	XM_014199797.1	mitochondrial import receptor subunit TOM5 homolog	1.53
LOC106581122	XM_014162880.1	lebercilin-like protein	1.53
pde6d	XM_014168616.1	phosphodiesterase 6D%2C cGMP-specific%2C rod%2C delta	1.53
LOC106560579	XM_014123592.1	retinal rod rhodopsin-sensitive cGMP 3'%2C5'-cyclic phosphodiesterase subunit delta	1.53
ndub4	NM_001141168.1	NADH dehydrogenase 1 beta subcomplex subunit 4	1.53
LOC106610070	XM_014209209.1	parvalbumin%2C thymic CPV3-like	1.53
LOC106603453	XM_014197161.1	derlin-2-like	1.53
LOC106607688	XM_014204900.1	cytochrome c oxidase subunit 7A2%2C mitochondrial	1.53
LOC106598901	XM_014189920.1	15 kDa selenoprotein-like	1.53
LOC106601894	XM_014194324.1	jmjC domain-containing protein 8-like	1.53
LOC106603327	XM_014196867.1	non-histone chromosomal protein HMG-14A-like	1.53
LOC106564190	XM_014130228.1	DBIRD complex subunit ZNF326-like	1.53
LOC106602281	XM_014194812.1	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6-like	1.53
LOC106605008	XM_014200198.1	stathmin-like	1.53
LOC106572941	XM_014147534.1	neuropeptides B/W receptor type 2-like	1.53
LOC106600926	XM_014192717.1	protein PET100 homolog%2C mitochondrial-like	1.52
cplx1	NM_001140038.1	Complexin-1	1.52
LOC106576359	XM_014153479.1	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 2%2C mitochondrial-like	1.52
ube2v2	XM_014191134.1	ubiquitin-conjugating enzyme E2 variant 2	1.52

LOC106566142	XM_014133992.1	dynactin subunit 2-like	1.52
LOC106583191	XM_014167096.1	probable protein BRICK1	1.52
lyrm4	XM_014188368.1	LYR motif containing 4	1.52
LOC106592956	XM_014184308.1	general transcription factor IIE subunit 2-like	1.52
rs30	NM_001146588.1	40S ribosomal protein S30	1.52
LOC106571903	XM_014145450.1	platelet glycoprotein IX-like	1.52
LOC106589895	XM_014180309.1	oxidoreductase-like domain-containing protein 1	1.52
smim15	XM_014171061.1	small integral membrane protein 15	1.51
sfrs1	NM_001173643.1	Splicing factor%2C arginine/serine-rich 1	1.51
LOC106600955	XM_014192764.1	ferritin%2C middle subunit-like	1.51
ndufb3	NM_001146531.1	NADH dehydrogenase (ubiquinone) 1 beta subcomplex%2C 3%2C 12kDa	1.51
LOC106579764	XM_014159960.1	microsomal glutathione S-transferase 3-like	1.51
pagr1	XM_014159253.1	PAXIP1 associated glutamate-rich protein 1	1.51
pdyn	NM_001140923.1	prodynorphin	1.51
LOC106609400	XM_014208191.1	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 2%2C mitochondrial-like	1.51
LOC106563115	XM_014128385.1	ATPase inhibitor B%2C mitochondrial-like	1.51
LOC106562465	XM_014127362.1	signal peptidase complex catalytic subunit SEC11A	1.50
LOC106565436	XM_014132573.1	SAP domain-containing ribonucleoprotein-like	1.50
LOC106589582	XM_014179727.1	parvalbumin%2C thymic CPV3-like	1.50
dync2li1	XM_014210574.1	dynein%2C cytoplasmic 2%2C light intermediate chain 1	1.50
LOC106584719	XM_014170241.1	eukaryotic translation initiation factor 3 subunit J-A-like	1.50
borcs7	XM_014154003.1	BLOC-1 related complex subunit 7	1.50
LOC106607317	XM_014204160.1	formin-like protein 1	1.50
LOC106570481	XM_014142876.1	cytochrome b-c1 complex subunit 7-like	1.50
LOC106573564	XM_014148711.1	mitochondrial import inner membrane translocase subunit Tim13	1.50
ovca2	NM_001140808.1	ovarian tumor suppressor candidate 2	1.49
cssa13hxorf56	XM_014137510.1	chromosome ssa13 open reading frame%2C human CXorf56	1.49
LOC106591465	XM_014182702.1	transmembrane emp24 domain-containing protein 10-like	1.49
LOC106563619	XM_014129357.1	uncharacterized LOC106563619	1.49
LOC106567917	XM_014137806.1	non-histone chromosomal protein HMG-14-like	1.49
LOC106565916	XM_014133614.1	28S ribosomal protein S16%2C mitochondrial-like	1.49
comd5	NM_001140699.1	COMM domain-containing protein 5	1.49
LOC106609820	XM_014208845.1	transmembrane protein 60-like	1.49

dipa	NM_001146455.1	Delta-interacting protein A	1.49
pqbp1	NM_001146422.1	Polyglutamine-binding protein 1	1.49
LOC106585740	XM_014172313.1	uncharacterized HIT-like protein Synpcc7942_1390	1.49
LOC106585593	XM_014171978.1	ubiquitin-like protein 4A-A	1.49
txd17	NM_001141499.1	Thioredoxin domain-containing protein 17	1.48
LOC106600762	XM_014192379.1	ferritin%2C middle subunit-like	1.48
gtf2h5	NM_001141298.1	general transcription factor IIH%2C polypeptide 5	1.48
LOC106610435	XM_014209804.1	polyadenylate-binding protein-interacting protein 2B-like	1.48
LOC106601742	XM_014194098.1	NHP2-like protein 1	1.48
LOC106569998	XM_014141834.1	cytochrome c oxidase subunit 6B1-like	1.48
snrpc	XM_014146394.1	small nuclear ribonucleoprotein polypeptide C	1.48
rnasek	XM_014197441.1	ribonuclease%2C RNase K	1.48
LOC106585554	XM_014171905.1	transmembrane protein 230-like	1.48
LOC106569554	XM_014141019.1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13-like	1.48
LOC106577450	XM_014155436.1	structure-specific endonuclease subunit slx1-like	1.48
LOC106575072	XM_014151229.1	cyclin-dependent kinase inhibitor 1B-like	1.47
LOC106564768	XM_014131122.1	echinoderm microtubule-associated protein-like 6	1.47
LOC106589535	XM_014179635.1	cold shock domain-containing protein C2-like	1.47
LOC106561545	XM_014125619.1	fibroblast growth factor receptor substrate 2-like	1.47
LOC106608529	XM_014206486.1	bolA-like protein 2	1.47
LOC106610705	XM_014210203.1	ATPase inhibitor B%2C mitochondrial-like	1.47
LOC106603561	XM_014197413.1	non-histone chromosomal protein HMG-14-like	1.47
LOC106573600	XM_014148783.1	cold-inducible RNA-binding protein B-like	1.47
LOC106586675	XM_014174199.1	ubiquitin-conjugating enzyme E2 A-like	1.47
vps37d	XM_014197151.1	vacuolar protein sorting 37 homolog D (S. cerevisiae)	1.47
jmjd7	XM_014194092.1	jumonji domain containing 7	1.47
tm14c	NM_001141158.1	Transmembrane protein 14C	1.47
LOC106576117	XM_014153006.1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5-like	1.46
LOC100194563	NM_001139649.1	mitochondrial ribosomal protein L51-like	1.46
LOC106567898	XM_014137768.1	ribonuclease kappa-B	1.46
lin7b	NM_001146623.1	lin-7 homolog B (C. elegans)	1.46
LOC106590380	XM_014181352.1	GTP-binding protein Rheb-like	1.46
spcs3	NM_001140682.2	signal peptidase complex subunit 3 homolog (S. cerevisiae)	1.46

LOC106587964	XM_014176624.1	heat shock factor-binding protein 1-like	1.46
mrpl34	XM_014189988.1	mitochondrial ribosomal protein L34	1.46
LOC106569431	XM_014140782.1	NEDD8	1.46
cdc26	NM_001140845.1	cell division cycle 26	1.46
pfdn5	XM_014132914.1	prefoldin subunit 5	1.46
LOC106587940	XM_014176600.1	gamma-aminobutyric acid receptor-associated protein-like 2	1.46
LOC106562297	XM_014127080.1	COX assembly mitochondrial protein 2 homolog	1.46
LOC106580140	XM_014160858.1	cytochrome c oxidase subunit 7C%2C mitochondrial-like	1.46
LOC106588914	XM_014178461.1	cytochrome b-c1 complex subunit 7-like	1.46
LOC106568402	XM_014138714.1	activated RNA polymerase II transcriptional coactivator p15-like	1.46
LOC106584057	XM_014168878.1	protein mago nashi homolog	1.46
LOC106583097	XM_014166913.1	MICOS complex subunit Mic10-like	1.46
syub	NM_001141659.2	Beta-synuclein	1.46
ltv1l	NM_001139624.1	LTV1-like	1.46
ino80e	XM_014201017.1	INO80 complex subunit E	1.46
vps25	NM_001141281.1	vacuolar protein sorting 25 homolog (S. cerevisiae)	1.46
pr38a	NM_001141758.1	Pre-mRNA-splicing factor 38A	1.46
LOC106605822	XM_014201784.1	ubiquitin-like protein ATG12	1.46
LOC106603165	XM_014196473.1	cofilin-2-like	1.46
LOC106569530	XM_014140975.1	cytochrome b-c1 complex subunit 10	1.46
tmem234	XM_014210094.1	transmembrane protein 234	1.46
LOC106597037	XM_014188298.1	putative E3 ubiquitin-protein ligase UBR7	1.46
LOC106567640	XM_014137170.1	histone H2A	1.46
LOC106579227	XM_014158931.1	histone H3.3	1.46
cssa10h12orf73	XM_014124713.1	chromosome ssa10 open reading frame%2C human C12orf73	1.45
LOC106603170	XM_014196484.1	FUN14 domain-containing protein 1A-like	1.45
sc11a	NM_001141078.1	Signal peptidase complex catalytic subunit SEC11A	1.45
LOC106593927	XM_014185304.1	uncharacterized LOC106593927	1.45
LOC106579885	XM_014160216.1	transmembrane protein 230-like	1.45
LOC106583751	XM_014168307.1	mesencephalic astrocyte-derived neurotrophic factor-like	1.45
nr13	XM_014127370.1	Anti-apoptotic protein NR13	1.45
LOC106587952	XM_014176616.1	proteasome subunit beta type-3	1.45
LOC106583964	XM_014168686.1	prothymosin alpha-A-like	1.45

LOC106566149	XM_014134004.1	methionine--tRNA ligase%2C cytoplasmic-like	1.45
hs020	NM_001146519.1	HSPC020 homolog	1.45
LOC106573260	XM_014148168.1	protein LSM14 homolog A-like	1.45
mmab	XM_014160778.1	methylmalonic aciduria (cobalamin deficiency) cblB type	1.45
LOC106613708	XM_014216245.1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 11-like	1.45
LOC106577644	XM_014155944.1	mitochondrial fission 1 protein-like	1.45
LOC106610231	XM_014209448.1	U6 snRNA-associated Sm-like protein LSm6	1.45
comd1	NM_001140257.1	COMM domain-containing protein 1	1.45
LOC106585051	XM_014170807.1	ubiquitin fusion degradation protein 1 homolog	1.45
LOC106608492	XM_014206429.1	uncharacterized LOC106608492	1.45
hsp10	XM_014165255.1	heat shock protein 10	1.45
LOC106587672	XM_014176256.1	uncharacterized LOC106587672	1.45
LOC106609477	XM_014208350.1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12-like	1.45
adat2	XM_014144503.1	adenosine deaminase%2C tRNA-specific 2	1.45
LOC106564829	XM_014131264.1	mitochondrial import receptor subunit TOM20 homolog	1.44
atp5g2	XM_014165625.1	ATP synthase%2C H+ transporting%2C mitochondrial F0 complex%2C subunit c-2	1.44
LOC106561701	XM_014125913.1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8-like	1.44
LOC106581181	XM_014163061.1	dynein light chain 2%2C cytoplasmic	1.44
mettl3	XM_014156116.1	methyltransferase like 3	1.44
LOC106608971	XM_014207248.1	H/ACA ribonucleoprotein complex subunit 3	1.44
LOC106613740	XM_014216304.1	cold-inducible RNA-binding protein B-like	1.44
LOC106561931	XM_014126337.1	ARL14 effector protein-like	1.44
LOC106607035	XM_014203593.1	uncharacterized protein C19orf43-like	1.44
LOC106612309	XM_014213353.1	HIG1 domain family member 2A-like	1.44
LOC106588347	XM_014177235.1	protein AF1q-like	1.44
LOC106579190	XM_014158859.1	V-type proton ATPase 16 kDa proteolipid subunit	1.44
LOC106609683	XM_014208655.1	26S protease regulatory subunit 7	1.44
LOC106577802	XM_014156158.1	NEDD8-like	1.44
alg9	XM_014198422.1	ALG9%2C alpha-1%2C2-mannosyltransferase	1.44
LOC106611995	XM_014212750.1	ras-related protein Rab-33A-like	1.44
LOC106601332	XM_014193463.1	uncharacterized protein C19orf43-like	1.44
LOC106582252	XM_014165136.1	60S ribosomal protein L31	1.44
mrpl10	XM_014159129.1	mitochondrial ribosomal protein L10	1.44

psme3	XM_014158502.1	proteasome activator subunit 3	1.44
LOC106600912	XM_014192682.1	gastrula zinc finger protein xFG20-1-like	1.44
LOC106563057	XM_014128278.1	cytochrome c oxidase subunit 5B%2C mitochondrial-like	1.44
pold4	NM_001146625.1	polymerase (DNA-directed)%2C delta 4%2C accessory subunit	1.44
t4s5	NM_001141538.1	Transmembrane 4 L6 family member 5	1.43
eif3g	NM_001140944.1	eukaryotic translation initiation factor 3%2C subunit G	1.43
LOC106590149	XM_014180800.1	charged multivesicular body protein 5	1.43
abracl	XM_014204735.1	ABRA C-terminal like	1.43
LOC106604953	XM_014200117.1	histone deacetylase 3	1.43
pfd2	NM_001141000.2	Prefoldin subunit 2	1.43
LOC106567989	XM_014137953.1	cytochrome c oxidase subunit 5B%2C mitochondrial-like	1.43
ube2c	XM_014168153.1	ubiquitin-conjugating enzyme E2C	1.43
LOC106582960	XM_014166607.1	mRNA turnover protein 4 homolog	1.43
LOC106574023	XM_014149533.1	transcription factor BTF3 homolog 4	1.43
LOC106578873	XM_014158110.1	histone H3.3	1.43
LOC106567817	XM_014137580.1	uncharacterized LOC106567817	1.43
LOC106583697	XM_014168196.1	cytochrome c oxidase assembly protein COX14 homolog	1.43
LOC106577457	XM_014155453.1	60S ribosomal protein L34	1.43
tcp4	NM_001141005.2	Activated RNA polymerase II transcriptional coactivator p15	1.43
LOC106578447	XM_014157248.1	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4-like	1.43
pgrmc1	NM_001146359.1	progesterone receptor membrane component 1	1.43
LOC106611239	XM_014211237.1	proteasome subunit alpha type-3	1.43
aurkaip1	NM_001141133.1	aurora kinase A interacting protein 1	1.42
LOC106568316	XM_014138546.1	mitotic-spindle organizing protein 2-like	1.42
LOC106587263	XM_014175499.1	embryonic polyadenylate-binding protein 2-B-like	1.42
LOC106608255	XM_014206107.1	ER membrane protein complex subunit 7-like	1.42
LOC106568198	XM_014138326.1	cytochrome b-c1 complex subunit 9-like	1.42
LOC106562909	XM_014127997.1	28S ribosomal protein S24%2C mitochondrial-like	1.42
ndua4	NM_001146612.1	NADH dehydrogenase 1 alpha subcomplex subunit 4	1.42
i2c2	NM_001141115.1	Eukaryotic translation initiation factor 2C 2	1.42
LOC106582745	XM_014166132.1	mitochondrial import inner membrane translocase subunit Tim17-A	1.42
cssa24h22orf39	XM_014170809.1	chromosome ssa24 open reading frame%2C human C22orf39	1.42
fam32a	NM_001140918.1	family with sequence similarity 32%2C member A	1.42

LOC106605735	XM_014201635.1	inhibitor of growth protein 4-like	1.42
LOC106574050	XM_014149576.1	zinc finger protein 287-like	1.42
ramp3	NM_001141893.1	Receptor activity-modifying protein 3	1.42
ck046	XM_014174760.1	CK046 protein	1.42
wbscr22	XM_014215407.1	Williams Beuren syndrome chromosome region 22	1.42
LOC106585463	XM_014171689.1	small nuclear ribonucleoprotein Sm D3-like	1.42
LOC106584048	XM_014168858.1	UPF0690 protein C1orf52 homolog	1.42
LOC106604860	XM_014199931.1	zinc finger protein 432-like	1.42
dyl1	NM_001141596.1	Dynein light chain 1%2C cytoplasmic	1.41
LOC106581973	XM_014164576.1	DCN1-like protein 2	1.41
LOC106577556	XM_014155699.1	eukaryotic translation initiation factor 5A-1	1.41
LOC106563983	XM_014129939.1	actin-related protein 2/3 complex subunit 1A-like	1.41
LOC106563483	XM_014129114.1	barrier-to-autointegration factor-like	1.41
frih	NM_001146488.1	Ferritin%2C heavy subunit	1.41
LOC106581107	XM_014162851.1	small nuclear ribonucleoprotein Sm D2	1.41
LOC106563745	XM_014129578.1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2-like	1.41
LOC106603050	XM_014196186.1	transgelin-3-like	1.41
LOC106591262	XM_014182479.1	uncharacterized LOC106591262	1.41
LOC106580809	XM_014162266.1	neuromodulin-like	1.41
cssa07h4orf48	NM_001140874.1	chromosome ssa07 open reading frame%2C human C4orf48	1.41
LOC106601603	XM_014193936.1	cytochrome c oxidase subunit NDUFA4-like	1.41
LOC106580840	XM_014162319.1	histone H3.3	1.41
LOC106568726	XM_014139313.1	plasminogen activator inhibitor 1 RNA-binding protein-like	1.41
LOC106565348	XM_014132347.1	serine/arginine-rich splicing factor 3-like	1.41
djb12	NM_001140370.1	DnaJ homolog subfamily B member 12	1.41
LOC106562262	XM_014127029.1	methenyltetrahydrofolate synthase domain-containing protein-like	1.41
LOC106588646	XM_014177828.1	stathmin-like	1.41
LOC106581902	XM_014164346.1	fibrous sheath CABYR-binding protein-like	1.41
LOC106611705	XM_014212192.1	ubiquitin-conjugating enzyme E2 D2-like	1.41
mrps6	XM_014157989.1	mitochondrial ribosomal protein S6	1.40
pgls	NM_001146596.1	6-phosphogluconolactonase	1.40
LOC106579096	XM_014158646.1	26S protease regulatory subunit 8	1.40
LOC106573541	XM_014148670.1	39S ribosomal protein L54%2C mitochondrial-like	1.40

LOC106593758	XM_014185135.1	THUMP domain-containing protein 1-like	1.40
LOC106583886	XM_014168511.1	V-type proton ATPase 16 kDa proteolipid subunit	1.40
ube2e3	XM_014173635.1	ubiquitin-conjugating enzyme E2E 3 (UBC4/5 homolog%2C yeast)	1.40
timm9	XM_014194728.1	translocase of inner mitochondrial membrane 9 homolog (yeast)	1.40
LOC106609414	XM_014208217.1	28 kDa heat- and acid-stable phosphoprotein-like	1.40
rbm8a	NM_001141696.1	RNA binding motif protein 8A	1.40
LOC106571302	XM_014144212.1	nucleoporin Nup43-like	1.40
tceb1	XM_014159901.1	transcription elongation factor B (SIII)%2C polypeptide 1 (15kDa%2C elongin C)	1.40
LOC106587995	XM_014176656.1	transcription initiation factor IIA subunit 2	1.40
LOC106573597	XM_014148774.1	cold-inducible RNA-binding protein B-like	1.40
LOC106589607	XM_014179790.1	NHP2-like protein 1	1.40
mtap	NM_001140147.1	methylthioadenosine phosphorylase	1.40
LOC106612087	XM_014212927.1	UBX domain-containing protein 1-like	1.40
LOC106600830	XM_014192534.1	ATP synthase F(0) complex subunit C3%2C mitochondrial-like	1.40
LOC106589005	XM_014178626.1	proteasome subunit alpha type-2-like	1.40
psmb6	XM_014128982.1	proteasome subunit beta 6	1.40
LOC106570752	XM_014143238.1	OTU domain-containing protein 6B-like	1.40
LOC106612273	XM_014213289.1	DNA-directed RNA polymerase II subunit RPB7	1.40
LOC106601222	XM_014193251.1	DNA-directed RNA polymerases I%2C II%2C and III subunit RPABC2	1.40
ppp1cb	NM_001141688.1	protein phosphatase 1%2C catalytic subunit%2C beta isoform	1.40
LOC106587370	XM_014175713.1	cytochrome c oxidase subunit 4 isoform 1%2C mitochondrial-like	1.40
sdhaf2	XM_014175961.1	succinate dehydrogenase complex assembly factor 2	1.40
LOC106604523	XM_014199210.1	CDK2-associated and cullin domain-containing protein 1-like	1.40
gbp	NM_001279048.1	GSK-3-binding protein	1.40
LOC106590036	XM_014180529.1	zinc finger CCCH domain-containing protein 15-like	1.40
LOC106599988	XM_014191321.1	splicing factor 3A subunit 2-like	1.40
LOC106594183	XM_014185549.1	tax1-binding protein 1 homolog B-like	1.40
LOC106563255	XM_014128673.1	endothelial differentiation-related factor 1 homolog	1.40
mrps17	XM_014195738.1	mitochondrial ribosomal protein S17	1.40
LOC106589850	XM_014180250.1	39S ribosomal protein L27%2C mitochondrial-like	1.39
rbm17	XM_014207890.1	RNA binding motif protein 17	1.39
LOC100194632	NM_001139717.1	endothelial differentiation-related factor 1-1	1.39
commd6	NM_001141062.1	COMM domain containing 6	1.39

LOC106609143	XM_014207621.1	ragulator complex protein LAMTOR4	1.39
cnbp	NM_001139745.1	CCHC-type zinc finger%2C nucleic acid binding protein	1.39
LOC106566440	XM_014134478.1	peptidyl-prolyl cis-trans isomerase FKBP1A-like	1.39
LOC106596537	XM_014187819.1	U6 snRNA-associated Sm-like protein LSm8	1.39
LOC106582700	XM_014166019.1	U1 small nuclear ribonucleoprotein C-like	1.39
pfd1	NM_001141510.1	Prefoldin subunit 1	1.39
LOC106607684	XM_014204888.1	N-alpha-acetyltransferase 20-like	1.39
md19b	NM_001146654.1	Mediator of RNA polymerase II transcription subunit 19-B	1.39
LOC106568761	XM_014139399.1	UMP-CMP kinase	1.39
mfap1	XM_014127584.1	microfibrillar-associated protein 1	1.39
prpf18	XM_014124963.1	pre-mRNA processing factor 18	1.39
LOC106578297	XM_014156968.1	V-type proton ATPase 16 kDa proteolipid subunit	1.39
LOC100136564	NM_001123657.1	ferritin heavy subunit	1.39
mea1	XM_014154081.1	male-enhanced antigen 1	1.39
LOC106587425	XM_014175803.1	probable ribosome biogenesis protein RLP24	1.39
gbrap	NM_001142717.1	Gamma-aminobutyric acid receptor-associated protein	1.39
chac1	NM_001140335.1	ChaC%2C cation transport regulator-like 1	1.39
meaf6	XM_014142533.1	MYST/Esa1-associated factor 6	1.39
rbx1	NM_001123564.1	ring-box 1	1.39
tma20	NM_001141426.1	Translation machinery-associated protein 20	1.39
LOC106579473	XM_014159414.1	eukaryotic translation initiation factor 1A%2C X-chromosomal	1.39
gtpba	XM_014202036.1	GTP-binding protein 10	1.39
LOC106568863	XM_014139604.1	protein FAM131A-like	1.39
twf1	NM_001140132.1	Twinfilin-1	1.39
LOC106590604	XM_014181724.1	proteasome maturation protein-like	1.39
LOC106566794	XM_014135189.1	transforming protein RhoA-like	1.38
LOC106610772	XM_014210331.1	cytochrome c oxidase subunit 7A2%2C mitochondrial-like	1.38
LOC106599031	XM_014190084.1	ubiquitin-60S ribosomal protein L40-like	1.38
LOC106602047	XM_014194496.1	ATP synthase subunit d%2C mitochondrial-like	1.38
LOC106588193	XM_014176940.1	ATPase inhibitor A%2C mitochondrial-like	1.38
sap18	NM_001279001.1	Histone deacetylase complex subunit SAP18	1.38
LOC106567406	XM_014136601.1	complement component 1 Q subcomponent-binding protein%2C mitochondrial-like	1.38
eif4e2	XM_014192045.1	eukaryotic translation initiation factor 4E family member 2	1.38

LOC106611512	XM_014211791.1	ubiquitin-conjugating enzyme E2 A	1.38
LOC106612297	XM_014213339.1	glutathione peroxidase 3-like	1.38
LOC106562065	XM_014126614.1	embryonic polyadenylate-binding protein 2-B-like	1.38
LOC106565035	XM_014131620.1	cytochrome c oxidase subunit 6C-1	1.38
LOC106567767	XM_014137483.1	non-POU domain-containing octamer-binding protein-like	1.38
LOC106611785	XM_014212367.1	small integral membrane protein 19-like	1.38
fkb1b	NM_001141565.2	FK506-binding protein 1B	1.38
inip	XM_014147377.1	INTS3 and NABP interacting protein	1.38
LOC106606979	XM_014203510.1	THO complex subunit 4-like	1.38
LOC106562230	XM_014126959.1	cleavage and polyadenylation specificity factor subunit 5-like	1.38
acbd7	NM_001141117.1	acyl-CoA binding domain containing 7	1.38
atp5h	NM_001139687.1	ATP synthase%2C H+ transporting%2C mitochondrial F0 complex%2C subunit d	1.38
commd10	NM_001146382.1	COMM domain containing 10	1.38
LOC106598624	XM_014189722.1	galectin-related protein B-like	1.38
LOC106606918	XM_014203387.1	ER membrane protein complex subunit 10-like	1.38
rwdd4	XM_014206481.1	RWD domain containing 4	1.38
LOC106593029	XM_014184373.1	cyclin-dependent kinase 5 activator 2-like	1.38
LOC106566500	XM_014134587.1	ER membrane protein complex subunit 3-like	1.38
ift22	NM_001140751.1	intraflagellar transport 22	1.38
fkbp3	NM_001141274.1	FK506 binding protein 3	1.38
sae1	NM_001146514.1	SUMO1 activating enzyme subunit 1	1.38
ppia	NM_001146606.1	peptidylprolyl isomerase A (cyclophilin A)	1.38
LOC106585258	XM_014171268.1	eukaryotic translation initiation factor 4E-binding protein 2-like	1.38
LOC106574824	XM_014150915.1	PEST proteolytic signal-containing nuclear protein-like	1.38
LOC106567425	XM_014136661.1	tetratricopeptide repeat protein 1-like	1.38
LOC106577413	XM_014155380.1	fumarate hydratase%2C mitochondrial	1.38
LOC106563496	XM_014129146.1	peptidyl-prolyl cis-trans isomerase FKBP2-like	1.38
LOC106563497	XM_014129147.1	protein phosphatase 1 regulatory subunit 14B-like	1.38
LOC106562224	XM_014126951.1	probable ribosome biogenesis protein RLP24	1.38
ddrgk1	XM_014132927.1	DDRGK domain containing 1	1.38
LOC106576246	XM_014153314.1	mitochondrial inner membrane protease ATP23 homolog	1.38
atp5g3	XM_014172926.1	ATP synthase%2C H+ transporting%2C mitochondrial F0 complex%2C subunit c-3	1.38
LOC106562614	XM_014127569.1	mortality factor 4-like protein 1	1.38

LOC106578266	XM_014156930.1	SUMO-conjugating enzyme UBC9-B-like	1.38
grl1	NM_001140623.1	Gamma-aminobutyric acid receptor-associated protein-like 1	1.37
LOC106587585	XM_014176115.1	39S ribosomal protein L4%2C mitochondrial-like	1.37
LOC106610898	XM_014210565.1	pre-mRNA-splicing factor syf2-like	1.37
gabarapl2	XM_014148919.1	GABA(A) receptor-associated protein like 2	1.37
LOC106610623	XM_014210040.1	uncharacterized protein C15orf57 homolog	1.37
nudc	NM_001146614.2	nuclear distribution gene C homolog	1.37
LOC106613943	XM_014216789.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1-like	1.37
LOC106607599	XM_014204688.1	proteasome subunit alpha type-6	1.37
LOC106581268	XM_014163224.1	28S ribosomal protein S23%2C mitochondrial-like	1.37
LOC106584850	XM_014170455.1	dnaJ homolog subfamily A member 4-like	1.37
LOC106608115	XM_014205835.1	splicing factor 3B subunit 6	1.37
commd7	NM_001141365.1	COMM domain containing 7	1.37
LOC106563505	XM_014129157.1	cofilin-2-like	1.37
anp32b	XM_014209536.1	acidic (leucine-rich) nuclear phosphoprotein 32 family%2C member B	1.37
s7a6o	XM_014126558.1	SLC7A6OS	1.37
LOC106603799	XM_014197914.1	small nuclear ribonucleoprotein Sm D2-like	1.37
LOC106608669	XM_014206761.1	G protein pathway suppressor 2-like	1.37
LOC106563029	XM_014128235.1	V-type proton ATPase subunit G 1-like	1.37
LOC106606648	XM_014202992.1	protein YIPF4	1.37
mo4l1	NM_001146521.1	Mortality factor 4-like protein 1	1.37
stmn4	XM_014192519.1	stathmin-like 4	1.37
mrpl16	XM_014151177.1	mitochondrial ribosomal protein L16	1.37
LOC106566962	XM_014135660.1	transcription initiation factor TFIID subunit 10-like	1.37
lerl1	XM_014199416.1	Leptin receptor overlapping transcript-like 1	1.37
sar1a	NM_001141789.1	SAR1 gene homolog A (S. cerevisiae)	1.37
LOC106595829	XM_014187174.1	uncharacterized protein KIAA1143 homolog	1.36
nduba	NM_001141557.1	NADH dehydrogenase 1 beta subcomplex subunit 10	1.36
LOC106572706	XM_014147111.1	N-alpha-acetyltransferase 10-like	1.36
LOC106588698	XM_014177962.1	mediator of RNA polymerase II transcription subunit 10	1.36
LOC106573579	XM_014148732.1	phospholipid hydroperoxide glutathione peroxidase%2C mitochondrial-like	1.36
LOC106585845	XM_014172529.1	transmembrane emp24 domain-containing protein 7-like	1.36
kpra	XM_014131117.1	Phosphoribosyl pyrophosphate synthetase-associated protein 1	1.36

crip2	NM_001146545.1	cysteine-rich protein 2	1.36
LOC106563671	XM_014129454.1	high mobility group protein B3-like	1.36
LOC106613902	XM_014216652.1	calreticulin-like	1.36
LOC106592861	XM_014184209.1	deoxyhypusine synthase-like	1.36
djc18	NM_001165308.1	DnaJ homolog subfamily C member 18	1.36
ub2v1	NM_001140875.1	Ubiquitin-conjugating enzyme E2 variant 1	1.36
LOC106609785	XM_014208797.1	nuclear-interacting partner of ALK-like	1.36
arpc3	NM_001142718.1	actin related protein 2/3 complex%2C subunit 3	1.36
LOC106609717	XM_014208689.1	DNA-(apurinic or apyrimidinic site) lyase-like	1.36
snf8	XM_014179044.1	SNF8%2C ESCRT-II complex subunit	1.36
LOC106601210	XM_014193231.1	heme-binding protein 1-like	1.36
LOC106582216	XM_014165071.1	BTB/POZ domain-containing protein KCTD4-like	1.36
LOC106571095	XM_014143764.1	ER membrane protein complex subunit 7-like	1.36
LOC106566721	XM_014135038.1	prostaglandin E synthase 3-like	1.36
LOC106603926	XM_014198178.1	heterogeneous nuclear ribonucleoprotein A/B-like	1.36
LOC106588845	XM_014178326.1	chromobox protein homolog 3-like	1.36
LOC106592831	XM_014184177.1	COX assembly mitochondrial protein homolog	1.36
commd9	NM_001141001.1	COMM domain containing 9	1.36
LOC106560679	XM_014123786.1	ATP synthase subunit g%2C mitochondrial-like	1.36
wdr70	XM_014130171.1	WD repeat domain 70	1.36
pop7	XM_014206985.1	POP7 homolog%2C ribonuclease P/MRP subunit	1.36
LOC106568019	XM_014138012.1	cytochrome c oxidase subunit 7B%2C mitochondrial-like	1.36
LOC106601696	XM_014194049.1	protein Tob1-like	1.36
LOC106589015	XM_014178644.1	chromatin modification-related protein MEAF6	1.36
LOC106608215	XM_014206046.1	V-type proton ATPase subunit D-like	1.36
LOC106589849	XM_014180249.1	serine/arginine-rich splicing factor 2-like	1.36
LOC106571313	XM_014144237.1	ribosome production factor 2 homolog	1.36
LOC106581011	XM_014162664.1	methylosome subunit pICln-like	1.36
LOC100196400	XM_014137639.1	protein CWC15 homolog	1.36
LOC106611761	XM_014212307.1	cofilin-2-like	1.35
LOC106599769	XM_014191080.1	serine/arginine-rich splicing factor 11-like	1.35
LOC106609405	XM_014208200.1	protein LLP homolog	1.35
fam133b	XM_014142764.1	family with sequence similarity 133%2C member B	1.35

LOC106605847	XM_014201823.1	splicing factor U2AF 65 kDa subunit-like	1.35
fam69a	XM_014123115.1	family with sequence similarity 69%2C member A	1.35
LOC106567218	XM_014136245.1	tumor protein D54-like	1.35
cops5	XM_014181414.1	COP9 signalosome subunit 5	1.35
LOC106570102	XM_014142107.1	uncharacterized LOC106570102	1.35
cc068	NM_001140764.1	CC068 protein	1.35
LOC106560853	XM_014124227.1	protein FAM192A-like	1.35
LOC106570228	XM_014142325.1	thioredoxin-like protein 4A	1.35
sels	NM_001140931.1	selenoprotein S	1.35
LOC106562058	XM_014126601.1	mitotic spindle-associated MMXD complex subunit MIP18-like	1.35
id2	XM_014210193.1	inhibitor of DNA binding 2%2C dominant negative helix-loop-helix protein	1.35
LOC106602900	XM_014195884.1	gamma-aminobutyric acid receptor-associated protein	1.35
LOC106604794	XM_014199838.1	protocadherin alpha-C2-like	1.35
emc4	NM_001141096.1	ER membrane protein complex subunit 4	1.35
LOC106567526	XM_014136881.1	S-phase kinase-associated protein 1	1.35
LOC106586704	XM_014174252.1	protein jagunal homolog 1-A-like	1.35
LOC106580350	XM_014161293.1	selenoprotein M-like	1.35
LOC106563108	XM_014128333.1	corticotropin-releasing factor-binding protein-like	1.35
LOC106584215	XM_014169252.1	proteasome subunit beta type-5-like	1.35
LOC106607492	XM_014204489.1	ATP synthase F(0) complex subunit C3%2C mitochondrial-like	1.35
bok	XM_014189908.1	BCL2-related ovarian killer	1.35
LOC106591015	XM_014182185.1	E3 ubiquitin-protein ligase RNF115-like	1.35
hexim	NM_001139959.1	HEXIM protein	1.35
denr	NM_001141283.1	density-regulated protein	1.35
LOC106607142	XM_014203767.1	DNA-directed RNA polymerases I%2C II%2C and III subunit RPABC2-like	1.34
LOC106564007	XM_014129967.1	gastrula zinc finger protein XlCGF57.1-like	1.34
LOC106570207	XM_014142291.1	uncharacterized LOC106570207	1.34
LOC106586318	XM_014173487.1	methylmalonic aciduria and homocystinuria type D protein%2C mitochondrial-like	1.34
psmd9	NM_001141185.1	proteasome (prosome%2C macropain) 26S subunit%2C non-ATPase%2C 9	1.34
cssa10h1orf52	NM_001141204.1	chromosome ssa10 open reading frame%2C human C1orf52	1.34
LOC106576269	XM_014153352.1	V-type proton ATPase subunit E 1-like	1.34
LOC106594500	XM_014185871.1	anaphase-promoting complex subunit 16	1.34
LOC106587420	XM_014175797.1	cleavage and polyadenylation specificity factor subunit 5-like	1.34

7b2	XM_014143772.1	Neuroendocrine protein 7B2	1.34
calm2	NM_001139713.1	calmodulin 2 (phosphorylase kinase%2C delta)	1.34
LOC106611558	XM_014211902.1	peptidyl-prolyl cis-trans isomerase D-like	1.34
LOC106602970	XM_014196045.1	Sjoegren syndrome/scleroderma autoantigen 1 homolog	1.34
LOC106565058	XM_014131679.1	eukaryotic translation initiation factor 2 subunit 2-like	1.34
LOC106573595	XM_014148772.1	yjeF N-terminal domain-containing protein 3-like	1.34
LOC106562286	XM_014127068.1	cytochrome c oxidase subunit 4 isoform 1%2C mitochondrial-like	1.34
LOC106569426	XM_014140774.1	proteasome subunit beta type-5-like	1.34
sfrs9	XM_014128542.1	Splicing factor%2C arginine/serine-rich 9	1.34
LOC106563585	XM_014129301.1	eukaryotic translation initiation factor 1A%2C X-chromosomal-like	1.34
LOC106589310	XM_014179106.1	proteasome activator complex subunit 3-like	1.34
LOC106598876	XM_014189895.1	ras-related protein Rab-6B-like	1.34
LOC106601335	XM_014193466.1	solute carrier family 25 member 38-B-like	1.34
slc25a11	XM_014156891.1	solute carrier family 25 (mitochondrial carrier%3B oxoglutarate carrier)%2C member 11	1.34
arpc5l	NM_001141375.1	actin related protein 2/3 complex%2C subunit 5-like	1.34
cssa11h9orf16	XM_014128744.1	chromosome ssa11 open reading frame%2C human C9orf16	1.34
LOC106609347	XM_014208082.1	nuclear transcription factor Y subunit beta-like	1.34
psmc1	XM_014211088.1	proteasome 26S subunit%2C ATPase 1	1.34
LOC106586159	XM_014173071.1	mitochondrial import receptor subunit TOM20 homolog	1.33
LOC106576424	XM_014153602.1	myotrophin	1.33
LOC106567919	XM_014137811.1	Purkinje cell protein 4-like	1.33
chga	XM_014145052.1	chromogranin A	1.33
LOC106576510	XM_014153727.1	prickle-like protein 1	1.33
LOC106580927	XM_014162485.1	succinate dehydrogenase [ubiquinone] cytochrome b small subunit B%2C mitochondrial-like	1.33
LOC106587003	XM_014174788.1	surfeit locus protein 4-like	1.33
LOC106589470	XM_014179498.1	WW domain-binding protein 2-like	1.33
cf105	NM_001141548.1	CF105 protein	1.33
LOC106593509	XM_014184850.1	DOMON domain-containing protein FRRS1L-like	1.33
LOC106574862	XM_014150979.1	synapse-associated protein 1-like	1.33
chchd6	XM_014132081.1	coiled-coil-helix-coiled-coil-helix domain containing 6	1.33
aarsd1	NM_001140214.1	alanyl-tRNA synthetase domain containing 1	1.33
LOC106575808	XM_014152500.1	retinoic acid-induced protein 2-like	1.33
cbx1	NM_001165285.1	chromobox homolog 1	1.33

LOC106613741	XM_014216308.1	cold-inducible RNA-binding protein B-like	1.33
LOC106571589	XM_014144872.1	uncharacterized LOC106571589	1.33
lsm7	XM_014141126.1	LSM7 homolog%2C U6 small nuclear RNA and mRNA degradation associated	1.33
taf11	NM_001141392.1	TAF11 RNA polymerase II%2C TATA box binding protein (TBP)-associated factor%2C 28kDa	1.33
LOC106576832	XM_014154285.1	gamma-aminobutyric acid receptor-associated protein-like 1	1.33
LOC106599148	XM_014190253.1	calreticulin-like	1.33
ube2g2	XM_014165761.1	ubiquitin-conjugating enzyme E2G 2	1.33
LOC106562977	XM_014128176.1	heterogeneous nuclear ribonucleoprotein R	1.33
LOC106587125	XM_014175169.1	hematological and neurological expressed 1 protein-like	1.33
LOC106592674	XM_014184011.1	zinc finger protein 330-like	1.33
prdx1	XM_014179684.1	peroxiredoxin 1	1.33
LOC106561718	XM_014125933.1	uncharacterized LOC106561718	1.33
neum	NM_001139802.1	Neuromodulin	1.33
ndka	NM_001141244.1	Nucleoside diphosphate kinase A	1.33
LOC106572179	XM_014146100.1	stathmin-3-like	1.33
LOC106609141	XM_014207620.1	V-type proton ATPase subunit F	1.33
lzic	XM_014145630.1	leucine zipper and CTNNBIP1 domain containing	1.33
LOC106600385	XM_014191691.1	peroxisomal biogenesis factor 19-like	1.33
ub2l3	XM_014138439.1	Ubiquitin-conjugating enzyme E2 L3	1.33
chst2	NM_001140092.1	Carbohydrate sulfotransferase 2	1.33
LOC106610994	XM_014210774.1	charged multivesicular body protein 3	1.32
LOC106567263	XM_014136363.1	cell division control protein 42 homolog	1.32
tsr3	XM_014158932.1	TSR3%2C 20S rRNA accumulation%2C homolog (S. cerevisiae)	1.32
ndufa3	NM_001141206.1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex%2C 3%2C 9kDa	1.32
LOC106568888	XM_014139671.1	calreticulin-like	1.32
yjefn3	XM_014216300.1	YjeF N-terminal domain containing 3	1.32
LOC106604708	XM_014199640.1	cofilin-2-like	1.32
ppia1	NM_001141689.1	2-peptidylprolyl isomerase A	1.32
LOC106565026	XM_014131599.1	dynein light chain roadblock-type 1	1.32
uba3	XM_014166581.1	ubiquitin-like modifier activating enzyme 3	1.32
LOC106565555	XM_014132813.1	actin-related protein 2/3 complex subunit 4	1.32
cfdp1	XM_014149074.1	craniofacial development protein 1	1.32
LOC106581067	XM_014162776.1	ribosome maturation protein SBDS	1.32

LOC106563281	XM_014128746.1	surfeit locus protein 4	1.32
ub2g1	XM_014133923.1	Ubiquitin-conjugating enzyme E2 G1	1.32
stml2	NM_001141736.1	Stomatin-like protein 2	1.32
LOC106601585	XM_014193896.1	transcription elongation factor B polypeptide 2	1.32
LOC106571686	XM_014145049.1	activator of 90 kDa heat shock protein ATPase homolog 1-like	1.32
dcps	XM_014197827.1	decapping enzyme%2C scavenger	1.32
LOC106570531	XM_014142960.1	transformer-2 protein homolog alpha-like	1.32
tbca	NM_001140987.1	tubulin folding cofactor A	1.32
LOC106567531	XM_014136892.1	GTP-binding protein SAR1a-like	1.32
LOC106573700	XM_014148990.1	heat shock factor-binding protein 1-like	1.32
u2af2	XM_014154451.1	U2 small nuclear RNA auxiliary factor 2	1.32
LOC106601215	XM_014193236.1	coiled-coil domain-containing protein 134-like	1.32
LOC106576015	XM_014152847.1	adenylyltransferase and sulfurtransferase MOCS3-like	1.32
tp4a1	NM_001165365.1	tyrosine phosphatase type IVA 1	1.32
LOC100136498	NM_001123609.1	macrophage migration inhibitory factor	1.32
LOC106601546	XM_014193839.1	uncharacterized LOC106601546	1.32
tppc3	XM_014178210.1	Trafficking protein particle complex subunit 3	1.32
LOC106585458	XM_014171683.1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1	1.32
LOC106593576	XM_014184926.1	conserved oligomeric Golgi complex subunit 1-like	1.32
fbxw12	XM_014145436.1	F-box and WD repeat domain containing 12	1.32
LOC106589537	XM_014179641.1	28 kDa heat- and acid-stable phosphoprotein-like	1.32
cf166	NM_001165297.1	CF166 protein	1.31
LOC106567495	XM_014136789.1	NEDD4 family-interacting protein 1-like	1.31
LOC106595987	XM_014187323.1	V-type proton ATPase subunit H-like	1.31
rab35	XM_014160050.1	RAB35%2C member RAS oncogene family	1.31
LOC106613487	XM_014215769.1	profilin-2-like	1.31
LOC106593403	XM_014184737.1	contactin-1-like	1.31
LOC106609277	XM_014207904.1	cell division cycle protein 123 homolog	1.31
LOC106576032	XM_014152852.1	ragulator complex protein LAMTOR4-like	1.31
LOC106591949	XM_014183220.1	guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-13-like	1.31
LOC106564619	XM_014130785.1	UPF0585 protein C16orf13 homolog A-like	1.31
LOC106571096	XM_014143765.1	KATNB1-like protein 1	1.31

ube2b	NM_001146361.1	ubiquitin-conjugating enzyme E2B (RAD6 homolog)	1.31
atp6v0c	XM_014192208.1	ATPase%2C H+ transporting%2C lysosomal 16kDa%2C V0 subunit c	1.31
mk67i	NM_001140700.1	MKI67 FHA domain-interacting nucleolar phosphoprotein-like	1.31
acp1	XM_014205655.1	acid phosphatase 1%2C soluble	1.31
surf1	NM_001141597.1	surfeit 1	1.31
LOC106573472	XM_014148558.1	casein kinase II subunit beta-like	1.31
LOC106576943	XM_014154490.1	splicing factor U2AF 65 kDa subunit-like	1.31
LOC106573420	XM_014148450.1	CD9 antigen-like	1.31
LOC106589821	XM_014180198.1	mitochondrial pyruvate carrier 1-like	1.31
LOC106577817	XM_014156173.1	thioredoxin-like	1.31
pebp1	NM_001141612.1	phosphatidylethanolamine binding protein 1	1.31
commd3	NM_001141012.1	COMM domain containing 3	1.31
LOC106580669	XM_014161976.1	eukaryotic translation initiation factor 4H-like	1.31
ube2v1	NM_001205110.1	ubiquitin-conjugating enzyme E2 variant 1	1.31
LOC106577557	XM_014155700.1	gamma-aminobutyric acid receptor-associated protein-like	1.31
LOC106585280	XM_014171334.1	arginine/serine-rich coiled-coil protein 2-like	1.31
LOC106612293	XM_014213328.1	serine/arginine-rich splicing factor 2-like	1.31
enp6	NM_001141709.1	Ectonucleoside triphosphate diphosphohydrolase 6	1.31
LOC106569985	XM_014141796.1	triosephosphate isomerase A	1.31
LOC100196748	XM_014150142.1	quinone oxidoreductase-like protein 1	1.31
mtpn	NM_001165386.1	myotrophin	1.31
ndufc1	XM_014212340.1	NADH dehydrogenase (ubiquinone) 1%2C subcomplex unknown%2C 1%2C 6kDa	1.30
LOC106580784	XM_014162192.1	14-3-3 protein epsilon-like	1.30
fam50a	NM_001146427.1	family with sequence similarity 50%2C member A	1.30
LOC106586370	XM_014173569.1	MOB-like protein phocein	1.30
LOC106590414	XM_014181416.1	ER membrane protein complex subunit 9-like	1.30
LOC106586850	XM_014174569.1	ice-structuring glycoprotein-like	1.30
LOC106582215	XM_014165068.1	translationally-controlled tumor protein homolog	1.30
LOC106610722	XM_014210239.1	14-3-3 protein beta/alpha-1-like	1.30
LOC106567415	XM_014136622.1	heterogeneous nuclear ribonucleoprotein A/B-like	1.30
LOC106609902	XM_014208950.1	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8%2C mitochondrial-like	1.30
polr2c	NM_001141873.1	polymerase (RNA) II (DNA directed) polypeptide C	1.30
LOC106604403	XM_014198988.1	ubiquitin-conjugating enzyme E2 D2	1.30

dap1	NM_001141583.1	Death-associated protein 1	1.30
LOC106564857	XM_014131322.1	malate dehydrogenase%2C cytoplasmic-like	1.30
fkbp1b	XM_014205706.1	FK506 binding protein 1B%2C 12.6 kDa	1.30
drg1	XM_014131454.1	developmentally regulated GTP binding protein 1	1.30
LOC106575588	XM_014152186.1	histone deacetylase complex subunit SAP18-like	1.30
LOC106584314	XM_014169422.1	acyl-CoA-binding domain-containing protein 6-like	1.30
LOC106611998	XM_014212752.1	E3 ubiquitin-protein ligase RNF4-like	1.30
ub2e3	XM_014165028.1	Ubiquitin-conjugating enzyme E2 E3	1.30
sox2	NM_001141718.1	SRY-box containing gene 2	1.30
LOC106583167	XM_014167033.1	class E basic helix-loop-helix protein 40-like	1.30
LOC106563719	XM_014129530.1	gamma-aminobutyric acid receptor subunit alpha-5-like	1.30
LOC106585454	XM_014171675.1	protein SET-like	1.30
LOC106563500	XM_014129151.1	calmodulin	1.30
saraf	XM_014209177.1	store-operated calcium entry-associated regulatory factor	1.30
ppme1	XM_014137585.1	protein phosphatase methylesterase 1	1.30
LOC106572212	XM_014146190.1	transcription initiation factor TFIID subunit 10-like	1.30
LOC106578494	XM_014157352.1	cytochrome c-type heme lyase-like	1.30
LOC106567379	XM_014136550.1	phosphatase and actin regulator 3-like	1.29
LOC106579942	XM_014160366.1	cytochrome c oxidase subunit 6A%2C mitochondrial	1.29
ube2i	XM_014158940.1	ubiquitin-conjugating enzyme E2I	1.29
pesc	NM_001139866.1	Pescadillo	1.29
naa38	XM_014155956.1	N(alpha)-acetyltransferase 38%2C NatC auxiliary subunit	1.29
LOC106574719	XM_014150735.1	paraspeckle component 1-like	1.29
mrps34	XM_014192203.1	mitochondrial ribosomal protein S34	1.29
LOC106603869	XM_014198073.1	neuron-specific protein family member 2-like	1.29
LOC106562632	XM_014127597.1	transmembrane emp24 domain-containing protein 2-like	1.29
LOC106593686	XM_014185058.1	guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-13-like	1.29
LOC106563531	XM_014129212.1	protein TSSC4-like	1.29
LOC106580428	XM_014161484.1	dynein light chain 1%2C cytoplasmic	1.29
hc127	XM_014198747.1	Hepatocellular carcinoma-associated antigen 127	1.29
LOC106582071	XM_014164790.1	ras-related protein Rab-5A-like	1.29
LOC106598823	XM_014189830.1	DCN1-like protein 1	1.29
LOC106562540	XM_014127480.1	myelin expression factor 2-like	1.29

LOC106574700	XM_014150711.1	cyclin-dependent kinase 5 activator 1-like	1.29
ik	NM_001173724.1	IK cytokine%2C down-regulator of HLA II	1.29
zn706	NM_001141044.1	Zinc finger protein 706	1.29
LOC106570595	XM_014143052.1	protein SET-like	1.29
LOC106579304	XM_014159111.1	charged multivesicular body protein 2a-like	1.29
LOC106612133	XM_014213037.1	heterogeneous nuclear ribonucleoprotein H-like	1.29
LOC106583084	XM_014166884.1	serine/arginine-rich splicing factor 3	1.29
LOC106577997	XM_014156512.1	heterogeneous nuclear ribonucleoprotein C-like	1.29
tmed2	XM_014161100.1	transmembrane emp24 domain trafficking protein 2	1.29
cssa16h11orf49	XM_014147803.1	chromosome ssa16 open reading frame%2C human C11orf49	1.29
LOC106606477	XM_014202702.1	core histone macro-H2A.2-like	1.28
LOC106589760	XM_014180087.1	mitoferrin-2-like	1.28
LOC106608224	XM_014206056.1	26S protease regulatory subunit 4-like	1.28
LOC106576223	XM_014153264.1	nuclear transcription factor Y subunit beta-like	1.28
LOC106569287	XM_014140478.1	nudC domain-containing protein 3-like	1.28
LOC106607186	XM_014203876.1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1-like	1.28
tmm32	NM_001141502.1	Transmembrane protein 32	1.28
lab	NM_001173766.1	Lupus La protein homolog B	1.28
LOC106562047	XM_014126588.1	heterogeneous nuclear ribonucleoprotein K-like	1.28
LOC106593495	XM_014184826.1	A-kinase anchor protein 8-like	1.28
LOC106561397	XM_014125339.1	ornithine decarboxylase antizyme 2-like	1.28
LOC106560291	XM_014123047.1	growth hormone-inducible transmembrane protein-like	1.28
LOC106579971	XM_014160440.1	protein SET	1.28
spf30	XM_014154464.1	Survival of motor neuron-related-splicing factor 30	1.28
LOC106588799	XM_014178233.1	mitochondrial import receptor subunit TOM7 homolog	1.28
stk25	NM_001140433.1	serine/threonine kinase 25	1.28
LOC106578562	XM_014157552.1	syntaxin-12-like	1.28
elob	NM_001141486.2	Transcription elongation factor B polypeptide 2	1.28
npm	NM_001140148.1	Nucleophosmin	1.28
LOC106607501	XM_014204503.1	eukaryotic translation initiation factor 1	1.28
LOC106600883	XM_014192633.1	SUMO-conjugating enzyme UBC9-like	1.28
LOC106584447	XM_014169800.1	protein QIL1-like	1.28

LOC106567593	XM_014137102.1	histone H3.3	1.28
LOC106564789	XM_014131165.1	apelin receptor B-like	1.28
LOC106600554	XM_014191997.1	glycine-rich RNA-binding protein-like	1.28
LOC106596773	XM_014188036.1	actin-related protein 2-A-like	1.28
ndufs3	XM_014175094.1	NADH dehydrogenase (ubiquinone) Fe-S protein 3%2C 30kDa (NADH-coenzyme Q reductase)	1.28
dyl2	NM_001141605.1	Dynein light chain 2%2C cytoplasmic	1.28
LOC106575630	XM_014152240.1	actin-related protein 2/3 complex subunit 2	1.28
arf3	XM_014135543.1	ADP-ribosylation factor 3	1.27
LOC106611763	XM_014212312.1	ubiquitin-like protein 3	1.27
LOC106585115	XM_014170960.1	nucleolysin TIA-1 isoform p40-like	1.27
mtx2	NM_001141391.2	metaxin 2	1.27
LOC106574227	XM_014149918.1	uncharacterized protein C7orf57 homolog	1.27
pdk1	XM_014173278.1	pyruvate dehydrogenase kinase%2C isozyme 1	1.27
LOC106571089	XM_014143757.1	protein max-like	1.27
LOC106567090	XM_014135974.1	tubulin alpha chain	1.27
LOC106565439	XM_014132580.1	tubulin alpha chain-like	1.27
LOC106601115	XM_014193052.1	pinin-like	1.27
LOC106611940	XM_014212628.1	heterogeneous nuclear ribonucleoprotein A/B-like	1.27
LOC106581816	XM_014164187.1	uncharacterized protein C7orf57-like	1.27
LOC106612439	XM_014213560.1	high mobility group-T protein-like	1.27
LOC106571940	XM_014145529.1	prostaglandin E synthase 3-like	1.27
ci025	XM_014160080.1	CI025 protein	1.27
LOC106583126	XM_014166959.1	F-box only protein 2-like	1.27
kad	NM_001139707.1	adenylate kinase 1-2	1.27
LOC106612793	XM_014214296.1	dual specificity protein phosphatase 14-like	1.27
LOC100194716	NM_001139761.1	selenoprotein T-like	1.27
rabggtb	NM_001140171.1	Rab geranylgeranyltransferase%2C beta subunit	1.27
LOC106561967	XM_014126460.1	mitochondrial carrier homolog 2-like	1.27
LOC106612606	XM_014213924.1	serine/arginine-rich splicing factor 7-like	1.27
tfpt	XM_014201661.1	TCF3 (E2A) fusion partner (in childhood Leukemia)	1.27
LOC106603705	XM_014197705.1	histone H3.3	1.27
chm1b	XM_014137435.1	Charged multivesicular body protein 1b	1.27
LOC106567639	XM_014137169.1	zinc finger protein ZPR1-like	1.27

LOC106569367	XM_014140676.1	heterogeneous nuclear ribonucleoprotein C-like	1.27
LOC106576148	XM_014153065.1	mesoderm-specific transcript homolog protein-like	1.27
LOC106601841	XM_014194245.1	heme oxygenase 2-like	1.27
LOC106581187	XM_014163083.1	transcriptional adapter 2-alpha-like	1.27
ub2r1	NM_001140115.1	Ubiquitin-conjugating enzyme E2 R1	1.27
cl012	NM_001146475.1	CL012 protein	1.27
taf7	NM_001140248.1	TAF7 RNA polymerase II%2C TATA box binding protein (TBP)-associated factor%2C 55kDa	1.27
LOC106607125	XM_014203741.1	WW domain-binding protein 2-like	1.27
LOC106580642	XM_014161919.1	high mobility group-T protein-like	1.27
LOC106564477	XM_014130591.1	programmed cell death protein 4-like	1.27
LOC106576681	XM_014153984.1	core histone macro-H2A.2-like	1.27
ppwd1	XM_014138550.1	peptidylprolyl isomerase domain and WD repeat containing 1	1.26
emc6	XM_014137940.1	ER membrane protein complex subunit 6	1.26
LOC100194664	XM_014204698.1	Vps20-associated 1 like 1	1.26
LOC106584722	XM_014170247.1	COP9 signalosome complex subunit 2-like	1.26
LOC106601417	XM_014193623.1	cerebellar degeneration-related protein 2-like	1.26
LOC106613600	XM_014216029.1	small glutamine-rich tetratricopeptide repeat-containing protein alpha-like	1.26
LOC106566691	XM_014134977.1	vesicle-associated membrane protein-associated protein B-like	1.26
LOC106603160	XM_014196468.1	calmodulin	1.26
ube2j1	NM_001139996.1	ubiquitin-conjugating enzyme E2%2C J1	1.26
pfn2	XM_014149743.1	profilin 2	1.26
LOC106579642	XM_014159747.1	gamma-soluble NSF attachment protein-like	1.26
pelo	XM_014158567.1	pelota homolog (Drosophila)	1.26
psd12	NM_001141769.1	26S proteasome non-ATPase regulatory subunit 12	1.26
ppp2r4	NM_001141756.1	protein phosphatase 2A activator%2C regulatory subunit 4	1.26
LOC106592767	XM_014184093.1	acidic leucine-rich nuclear phosphoprotein 32 family member E-like	1.26
LOC100194554	XM_014207488.1	solute carrier family 25 member 3	1.26
eef1e1	XM_014181051.1	eukaryotic translation elongation factor 1 epsilon 1	1.26
rnf34	XM_014130839.1	ring finger protein 34%2C E3 ubiquitin protein ligase	1.26
LOC106582630	XM_014165858.1	cytochrome c1%2C heme protein%2C mitochondrial-like	1.26
LOC106585705	XM_014172226.1	26S proteasome non-ATPase regulatory subunit 12-like	1.26
gmds	XM_014123207.1	GDP-mannose 4%2C6-dehydratase	1.25
hnrpg	XM_014199418.1	Heterogeneous nuclear ribonucleoprotein G	1.25

LOC106584440	XM_014169779.1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1-related-like	1.25
LOC106613829	XM_014216480.1	vesicle-trafficking protein SEC22b-B	1.25
LOC106584750	XM_014170292.1	transmembrane protein 178B-like	1.25
face1	NM_001140076.1	CAAX prenyl protease 1 homolog	1.25
LOC106575622	XM_014152233.1	FUN14 domain-containing protein 1-like	1.25
eif3j	XM_014175443.1	eukaryotic translation initiation factor 3%2C subunit J	1.25
LOC106580918	XM_014162474.1	mitochondrial fission factor homolog B-like	1.25
oaz1s	NM_001142707.1	ornithine decarboxylase antizyme 1S	1.25
LOC106571252	XM_014144067.1	14-3-3 protein zeta	1.25
LOC106605558	XM_014201344.1	platelet-activating factor acetylhydrolase IB subunit gamma-like	1.25
LOC106603817	XM_014197982.1	voltage-dependent anion-selective channel protein 1	1.25
hsf2	XM_014204746.1	heat shock factor 2	1.25
LOC106579884	XM_014160215.1	transmembrane protein 127-like	1.25
LOC106584153	XM_014169074.1	ubiquitin carboxyl-terminal hydrolase isozyme L5-like	1.25
LOC106599679	XM_014190972.1	beta-soluble NSF attachment protein-like	1.25
LOC100194523	NM_001139609.1	MGC81610 protein-like	1.25
LOC106578074	XM_014156653.1	polyadenylate-binding protein-interacting protein 2B-like	1.25
LOC106576826	XM_014154267.1	nucleolysin TIAR-like	1.25
ap3s1	XM_014160057.1	adaptor-related protein complex 3%2C sigma 1 subunit	1.24
cacybp	NM_001140952.1	calcyclin binding protein	1.24
LOC106576022	XM_014152821.1	serine-threonine kinase receptor-associated protein-like	1.24
LOC106610956	XM_014210682.1	eukaryotic translation initiation factor 5-like	1.24
renr	NM_001140091.1	Renin receptor	1.24
LOC106572481	XM_014146704.1	ubiquitin-conjugating enzyme E2 variant 1	1.24
LOC106571654	XM_014144965.1	cysteine-rich protein 2-like	1.24
LOC106612225	XM_014213199.1	NEDD4 family-interacting protein 1-like	1.24
LOC106567523	XM_014136870.1	voltage-dependent anion-selective channel protein 1-like	1.24
LOC106591329	XM_014182544.1	aspartate aminotransferase%2C cytoplasmic-like	1.24
LOC106593297	XM_014184635.1	ornithine aminotransferase%2C mitochondrial-like	1.24
LOC106590146	XM_014180790.1	myosin regulatory light polypeptide 9	1.24
prpf3	XM_014151195.1	pre-mRNA processing factor 3	1.24
LOC106612660	XM_014214050.1	14-3-3 protein epsilon-like	1.24

LOC106603856	XM_014198054.1	NEDD4 family-interacting protein 1-like	1.24
idh3a	XM_014208669.1	isocitrate dehydrogenase 3 (NAD+) alpha	1.23
LOC106600642	XM_014192145.1	phosphatidylinositol transfer protein beta isoform-like	1.23
LOC106583189	XM_014167094.1	ER membrane protein complex subunit 3	1.23
LOC106586437	XM_014173712.1	basic leucine zipper and W2 domain-containing protein 1-A-like	1.23
hnrnpk	XM_014172158.1	heterogeneous nuclear ribonucleoprotein K	1.23
LOC106583278	XM_014167248.1	tubulin alpha chain-like	1.23
rbtn1	NM_001146398.1	Rhombotin-1	1.23
LOC106578933	XM_014158211.1	protein phosphatase 1B-like	1.23
LOC106609126	XM_014207582.1	serine-threonine kinase receptor-associated protein-like	1.23
LOC106564670	XM_014130886.1	small ubiquitin-related modifier 2-like	1.23
LOC100380720	XM_014125643.1	F-actin-capping protein subunit alpha-2	1.23
LOC106574283	XM_014150081.1	acidic leucine-rich nuclear phosphoprotein 32 family member E-like	1.23
LOC106607800	XM_014205163.1	uncharacterized protein C1orf198 homolog	1.23
ap1m1	XM_014148669.1	adaptor-related protein complex 1%2C mu 1 subunit	1.23
LOC106610676	XM_014210147.1	protein phosphatase 1G-like	1.22
LOC106580853	XM_014162336.1	serine/arginine-rich splicing factor 7-like	1.22
ppp1cc	XM_014160404.1	protein phosphatase 1%2C catalytic subunit%2C gamma isozyme	1.22
LOC106571634	XM_014144936.1	serine/threonine-protein phosphatase PP1-beta catalytic subunit-like	1.22
actr8	NM_001140158.1	ARP8 actin-related protein 8 homolog (yeast)	1.22
LOC106608292	XM_014206155.1	transmembrane emp24 domain-containing protein 10-like	1.22
arf1	XM_014140595.1	ADP-ribosylation factor 1	1.22
LOC106605532	XM_014201296.1	protein LYRIC-like	1.21
cops3	NM_001140561.1	COP9 signalosome subunit 3	1.21
LOC106606270	XM_014202415.1	UPF0472 protein C16orf72 homolog	1.21
LOC100194681	XM_014200054.1	heterogeneous nuclear ribonucleoprotein A/B	1.21
LOC100380682	XM_014180902.1	protein CDV3 homolog	1.21
LOC106566654	XM_014134891.1	14-3-3 protein beta/alpha-2	1.20
LOC106566965	XM_014135663.1	TAR DNA-binding protein 43-like	1.20
LOC106567579	XM_014137062.1	14-3-3 protein epsilon-like	1.20
LOC106613688	XM_014216216.1	AP-1 complex subunit mu-1	1.19

Table S3. Gene Ontology (GO) categories with a significant ($p < 0.05$) overrepresentation of upregulated genes in Atlantic salmon parr subjected to an 8-week volitional exercise regime, compared to unexercised control fish.

GO ID	GO term	*p* value	No. genes in GO category	No. genes upregulated in GO category
GO:0016059	deactivation of rhodopsin mediated signaling	4.91E-05	24	8
GO:0018105	peptidyl-serine phosphorylation	0.000112069	332	28
GO:0008340	determination of adult lifespan	0.000262183	356	29
GO:0019722	calcium-mediated signaling	0.000280014	111	13
GO:0016056	rhodopsin mediated signaling pathway	0.000389687	68	8
GO:2000615	regulation of histone H3-K9 acetylation	0.000548256	38	9
GO:0048477	oogenesis	0.000557648	187	18
GO:0000185	activation of MAPKKK activity	0.000795386	32	6
GO:0072577	endothelial cell apoptotic process	0.000807135	20	6
GO:0051497	negative regulation of stress fiber assembly	0.000826275	88	12
GO:0010470	regulation of gastrulation	0.000953507	45	10
GO:0050803	regulation of synapse structure or activity	0.001047207	72	11
GO:0043966	histone H3 acetylation	0.001257902	129	16
GO:0042981	regulation of apoptotic process	0.00127063	245	21
GO:0010717	regulation of epithelial to mesenchymal transition	0.001361786	47	9
GO:0018107	peptidyl-threonine phosphorylation	0.001382254	177	18
GO:0044257	cellular protein catabolic process	0.001394764	29	7
GO:1903690	negative regulation of wound healing, spreading of epidermal cells	0.00143647	22	7
GO:0043984	histone H4-K16 acetylation	0.001548036	62	11
GO:0016458	gene silencing	0.001551823	44	10
GO:0046488	phosphatidylinositol metabolic process	0.001586795	52	9
GO:0090557	establishment of endothelial intestinal barrier	0.001887925	47	8
GO:0031023	microtubule organizing center organization	0.002296447	27	7
GO:0006987	activation of signaling protein activity involved in unfolded protein response	0.003059474	29	5
GO:0080182	histone H3-K4 trimethylation	0.003100813	34	9
GO:0007020	microtubule nucleation	0.003111667	47	8
GO:0090091	positive regulation of extracellular matrix disassembly	0.003166918	29	7

GO:0070983	dendrite guidance	0.003445746	30	9
GO:0070972	protein localization to endoplasmic reticulum	0.00350467	21	7
GO:0045927	positive regulation of growth	0.003686576	25	5
GO:0061136	regulation of proteasomal protein catabolic process	0.003688617	95	11
GO:0006903	vesicle targeting	0.003919159	41	7
GO:0006974	cellular response to DNA damage stimulus	0.004441235	291	23
GO:1901018	positive regulation of potassium ion transmembrane transporter activity	0.004441368	27	8
GO:2000599	NA	0.004603574	22	5
GO:2000021	regulation of ion homeostasis	0.00466305	24	7
GO:2000651	positive regulation of sodium ion transmembrane transporter activity	0.005544155	44	11
GO:0006338	chromatin remodeling	0.005792504	161	13
GO:0043052	thermotaxis	0.00583157	32	6
GO:0002385	mucosal immune response	0.006036644	21	5
GO:0043547	positive regulation of GTPase activity	0.006186397	732	49
GO:0051568	histone H3-K4 methylation	0.006212116	55	11
GO:0044255	cellular lipid metabolic process	0.006426544	120	13
GO:0034453	microtubule anchoring	0.006428213	31	7
GO:0086014	atrial cardiac muscle cell action potential	0.006746725	28	7
GO:0006306	DNA methylation	0.006752043	70	10
GO:0006513	protein monoubiquitination	0.007015936	91	9
GO:0007016	cytoskeletal anchoring at plasma membrane	0.00726445	68	13
GO:0048589	developmental growth	0.007643379	84	10
GO:0051482	positive regulation of cytosolic calcium ion concentration involved in phospholipase C-activating G-protein coupled signaling pathway	0.008130151	46	6
GO:0051225	spindle assembly	0.008557846	78	9
GO:2000648	positive regulation of stem cell proliferation	0.008945318	40	6
GO:0019827	stem cell population maintenance	0.008984222	156	13
GO:0043981	histone H4-K5 acetylation	0.009065338	48	7
GO:0007279	pole cell formation	0.00928416	20	4
GO:0043254	regulation of protein complex assembly	0.009417262	25	5
GO:2001259	positive regulation of cation channel activity	0.011040008	44	8
GO:1903428	positive regulation of reactive oxygen species biosynthetic process	0.01119157	22	4
GO:0043486	histone exchange	0.011332405	23	5

GO:2000026	regulation of multicellular organismal development	0.011381497	83	10
GO:0060562	epithelial tube morphogenesis	0.011412479	32	6
GO:0045200	establishment of neuroblast polarity	0.012094773	26	4
GO:0010458	exit from mitosis	0.01224387	39	6
GO:0032088	negative regulation of NF-kappaB transcription factor activity	0.012408966	154	11
GO:0006607	NLS-bearing protein import into nucleus	0.012946646	43	5
GO:0006302	double-strand break repair	0.013033434	98	8
GO:0046939	nucleotide phosphorylation	0.013283862	42	5
GO:0010288	response to lead ion	0.013649056	62	6
GO:0006342	chromatin silencing	0.013851989	167	14
GO:0043113	receptor clustering	0.014172273	76	10
GO:0019915	lipid storage	0.014244936	162	12
GO:0007052	mitotic spindle organization	0.014551215	181	13
GO:1902476	chloride transmembrane transport	0.014639653	138	10
GO:0048821	erythrocyte development	0.01485592	57	8
GO:0050821	protein stabilization	0.015980449	357	21
GO:0010634	positive regulation of epithelial cell migration	0.015985433	95	9
GO:0009299	mRNA transcription	0.016102279	27	4
GO:0014894	response to denervation involved in regulation of muscle adaptation	0.016185392	23	5
GO:0030948	negative regulation of vascular endothelial growth factor receptor signaling pathway	0.016458607	27	5
GO:0001932	regulation of protein phosphorylation	0.016551667	58	10
GO:0008654	phospholipid biosynthetic process	0.016566401	35	5
GO:0031345	negative regulation of cell projection organization	0.017073709	20	4
GO:0070555	response to interleukin-1	0.017195444	80	8
GO:0008104	protein localization	0.017735895	171	17
GO:0071420	cellular response to histamine	0.017741007	36	4
GO:0035159	regulation of tube length, open tracheal system	0.017817347	23	6
GO:0001702	gastrulation with mouth forming second	0.018119819	90	12
GO:0010960	magnesium ion homeostasis	0.018165753	35	7
GO:0006651	diacylglycerol biosynthetic process	0.018213974	30	5
GO:0038083	peptidyl-tyrosine autophosphorylation	0.01890937	64	7
GO:0090314	positive regulation of protein targeting to membrane	0.019883905	69	8

GO:0016573	histone acetylation	0.020072338	86	9
GO:0007608	sensory perception of smell	0.020123816	124	10
GO:0045454	cell redox homeostasis	0.020620634	102	11
GO:0051932	synaptic transmission, GABAergic	0.020887749	97	9
GO:0070317	negative regulation of G0 to G1 transition	0.020904017	20	4
GO:0048172	regulation of short-term neuronal synaptic plasticity	0.021181586	79	9
GO:0007095	mitotic G2 DNA damage checkpoint	0.021266996	130	9
GO:0060235	lens induction in camera-type eye	0.021501175	21	4
GO:0010310	regulation of hydrogen peroxide metabolic process	0.022132589	22	3
GO:0090162	establishment of epithelial cell polarity	0.022172154	61	7
GO:0097193	intrinsic apoptotic signaling pathway	0.022520883	128	8
GO:0032147	activation of protein kinase activity	0.022674737	185	12
GO:0045838	positive regulation of membrane potential	0.023160209	31	6
GO:0007186	G-protein coupled receptor signaling pathway	0.023378589	347	24
GO:0060024	rhythmic synaptic transmission	0.023523141	37	6
GO:0060996	dendritic spine development	0.02387406	48	5
GO:0090630	activation of GTPase activity	0.023951091	156	13
GO:0043409	negative regulation of MAPK cascade	0.024244472	26	5
GO:0034394	protein localization to cell surface	0.024291683	69	9
GO:0071709	membrane assembly	0.024944882	20	7
GO:0050729	positive regulation of inflammatory response	0.025255609	61	5
GO:0007214	gamma-aminobutyric acid signaling pathway	0.025291239	65	7
GO:0043030	regulation of macrophage activation	0.025349349	21	3
GO:0007611	learning or memory	0.025399332	138	13
GO:0007043	cell-cell junction assembly	0.025587908	37	5
GO:0007596	blood coagulation	0.026378668	460	24
GO:0001574	ganglioside biosynthetic process	0.026884891	28	3
GO:0045921	positive regulation of exocytosis	0.027109055	90	8
GO:0060397	JAK-STAT cascade involved in growth hormone signaling pathway	0.027703323	39	4
GO:0050832	defense response to fungus	0.027784826	83	11
GO:0021591	ventricular system development	0.027881717	65	10
GO:0043982	histone H4-K8 acetylation	0.027881868	46	6
GO:0006020	inositol metabolic process	0.027900196	26	4

GO:0030728	ovulation	0.02875822	23	5
GO:0071333	cellular response to glucose stimulus	0.028758386	168	11
GO:0055085	transmembrane transport	0.029664196	313	17
GO:0010592	positive regulation of lamellipodium assembly	0.029906142	76	6
GO:0034122	negative regulation of toll-like receptor signaling pathway	0.03009698	26	3
GO:0071158	positive regulation of cell cycle arrest	0.030418092	59	5
GO:0042129	regulation of T cell proliferation	0.030608072	26	3
GO:0032410	negative regulation of transporter activity	0.030627182	23	3
GO:0060307	regulation of ventricular cardiac muscle cell membrane repolarization	0.030926704	45	8
GO:0040013	negative regulation of locomotion	0.03097416	29	3
GO:0030183	B cell differentiation	0.031510451	107	10
GO:0032869	cellular response to insulin stimulus	0.031740473	248	15
GO:0048666	neuron development	0.031937239	143	13
GO:0008285	negative regulation of cell proliferation	0.032527749	855	47
GO:0048009	insulin-like growth factor receptor signaling pathway	0.032571413	67	7
GO:0000088	mitotic prophase	0.032664833	138	9
GO:1903861	positive regulation of dendrite extension	0.032969993	69	9
GO:0007420	brain development	0.034012426	572	32
GO:0015914	phospholipid transport	0.034064257	60	5
GO:0050796	regulation of insulin secretion	0.034293105	190	13
GO:0043968	histone H2A acetylation	0.034779066	31	4
GO:0007172	signal complex assembly	0.034794031	25	3
GO:0046627	negative regulation of insulin receptor signaling pathway	0.034896506	105	8
GO:0006468	protein phosphorylation	0.03552311	410	23
GO:0033120	positive regulation of RNA splicing	0.035602524	53	5
GO:0036336	dendritic cell migration	0.035627312	20	3
GO:1901017	negative regulation of potassium ion transmembrane transporter activity	0.035784643	22	3
GO:0050890	cognition	0.037377736	66	8
GO:0006979	response to oxidative stress	0.037391681	251	14
GO:1990090	cellular response to nerve growth factor stimulus	0.03748905	131	10
GO:1901800	positive regulation of proteasomal protein catabolic process	0.037789283	41	4
GO:0007617	mating behavior	0.038145761	42	4
GO:0042157	lipoprotein metabolic process	0.039195242	33	6

GO:0098910	regulation of atrial cardiac muscle cell action potential	0.039298313	20	7
GO:0046580	negative regulation of Ras protein signal transduction	0.039379957	78	7
GO:0050775	positive regulation of dendrite morphogenesis	0.039934217	80	8
GO:0050882	voluntary musculoskeletal movement	0.039940986	20	5
GO:0001881	receptor recycling	0.040266087	26	4
GO:0055072	iron ion homeostasis	0.040884551	65	7
GO:0035924	cellular response to vascular endothelial growth factor stimulus	0.040944957	77	7
GO:0051387	negative regulation of neurotrophin TRK receptor signaling pathway	0.041025891	26	4
GO:0031340	positive regulation of vesicle fusion	0.041064831	40	4
GO:1903779	regulation of cardiac conduction	0.041186252	24	4
GO:0021549	cerebellum development	0.041675869	178	12
GO:0009791	post-embryonic development	0.041987081	377	28
GO:0009628	response to abiotic stimulus	0.042114779	93	7
GO:0032922	circadian regulation of gene expression	0.042946404	194	14
GO:0030010	establishment of cell polarity	0.043382404	114	10
GO:0086005	ventricular cardiac muscle cell action potential	0.044153368	51	9
GO:0050727	regulation of inflammatory response	0.044379988	122	8
GO:0048536	spleen development	0.044516128	104	10
GO:0033148	positive regulation of intracellular estrogen receptor signaling pathway	0.044548004	48	7
GO:2001020	regulation of response to DNA damage stimulus	0.044873128	35	5
GO:0006779	porphyrin-containing compound biosynthetic process	0.044948131	28	7
GO:0051963	regulation of synapse assembly	0.045170262	54	7
GO:0006972	hyperosmotic response	0.045438982	76	5
GO:0007252	I-kappaB phosphorylation	0.046359992	31	4
GO:0001778	plasma membrane repair	0.046391858	20	4
GO:0045820	negative regulation of glycolytic process	0.046718094	42	4
GO:0042771	intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	0.046746549	74	7
GO:1902600	hydrogen ion transmembrane transport	0.047395413	90	5
GO:0031047	gene silencing by RNA	0.047534006	46	4
GO:0014066	regulation of phosphatidylinositol 3-kinase signaling	0.047712663	22	3
GO:0090307	mitotic spindle assembly	0.047937451	113	7
GO:0071374	cellular response to parathyroid hormone stimulus	0.048597204	33	4

GO:0070102	interleukin-6-mediated signaling pathway	0.049228778	24	3
GO:0035162	embryonic hemopoiesis	0.049881259	84	9
GO:0032482	Rab protein signal transduction	0.049913583	141	7
GO:0036010	protein localization to endosome	0.049996504	40	4

Table S4. Gene Ontology (GO) categories with a significant ($p < 0.05$) overrepresentation of downregulated genes in Atlantic salmon parr subjected to an 8-week volitional exercise regime, compared to unexercised control fish.

GO ID	GO term	*p* value	No. genes in GO category	No. genes downregulated in GO category
GO:0009792	embryo development ending in birth or egg hatching	8.48E-16	995	112
GO:0040035	hermaphrodite genitalia development	2.24E-10	401	52
GO:0000398	mRNA splicing, via spliceosome	5.41E-10	319	44
GO:0002119	nematode larval development	2.51E-09	812	83
GO:0016032	viral process	3.88E-09	647	60
GO:0040011	locomotion	6.52E-09	439	45
GO:0006369	termination of RNA polymerase II transcription	3.44E-08	140	27
GO:0048255	mRNA stabilization	3.06E-07	43	11
GO:0008340	determination of adult lifespan	7.05E-07	356	36
GO:0031124	mRNA 3'-end processing	9.06E-07	85	20
GO:0040020	regulation of meiotic nuclear division	6.65E-06	83	13
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	7.82E-06	58	18
GO:0000381	regulation of alternative mRNA splicing, via spliceosome	8.00E-06	175	21
GO:0007476	imaginal disc-derived wing morphogenesis	9.22E-06	115	13
GO:0022904	respiratory electron transport chain	1.03E-05	112	24
GO:0048025	negative regulation of mRNA splicing, via spliceosome	1.05E-05	107	17
GO:0006898	receptor-mediated endocytosis	1.18E-05	442	37
GO:0061025	membrane fusion	1.18E-05	75	14
GO:0000422	autophagy of mitochondrion	1.54E-05	79	14
GO:0010171	body morphogenesis	1.97E-05	241	29
GO:1902600	hydrogen ion transmembrane transport	2.03E-05	90	18
GO:0008286	insulin receptor signaling pathway	2.05E-05	654	43
GO:0006406	mRNA export from nucleus	2.19E-05	131	19
GO:0000394	RNA splicing, via endonucleolytic cleavage and ligation	2.29E-05	29	10
GO:0015991	ATP hydrolysis coupled proton transport	2.43E-05	53	11
GO:0018991	oviposition	4.19E-05	124	15
GO:0042776	mitochondrial ATP synthesis coupled proton transport	4.47E-05	31	11

GO:0006915	apoptotic process	6.15E-05	913	64
GO:0009409	response to cold	8.53E-05	122	15
GO:0006995	cellular response to nitrogen starvation	8.60E-05	35	9
GO:0035308	negative regulation of protein dephosphorylation	0.00012	24	6
GO:0034063	stress granule assembly	0.00013	39	7
GO:0007052	mitotic spindle organization	0.00014	181	18
GO:0002223	stimulatory C-type lectin receptor signaling pathway	0.00016	256	28
GO:0061001	regulation of dendritic spine morphogenesis	0.00017	62	6
GO:0070534	protein K63-linked ubiquitination	0.00017	118	15
GO:0043086	negative regulation of catalytic activity	0.00018	156	16
GO:0000413	protein peptidyl-prolyl isomerization	0.00019	61	12
GO:0035021	negative regulation of Rac protein signal transduction	0.00019	23	6
GO:0040024	dauer larval development	0.00022	32	6
GO:0040025	vulval development	0.00023	48	9
GO:0007294	germarium-derived oocyte fate determination	0.00024	23	5
GO:0070265	necrotic cell death	0.00026	23	5
GO:0070979	protein K11-linked ubiquitination	0.00039	69	12
GO:1900740	positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	0.00046	74	10
GO:0070936	protein K48-linked ubiquitination	0.00046	142	14
GO:0051437	positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	0.00049	162	24
GO:0006521	regulation of cellular amino acid metabolic process	0.00050	102	18
GO:0048208	COPII vesicle coating	0.00053	38	6
GO:0035196	production of miRNAs involved in gene silencing by miRNA	0.00065	27	6
GO:0034975	protein folding in endoplasmic reticulum	0.00066	40	7
GO:0038061	NIK/NF-kappaB signaling	0.00070	122	19
GO:0000723	telomere maintenance	0.00074	48	8
GO:0006367	transcription initiation from RNA polymerase II promoter	0.00081	478	32
GO:0017148	negative regulation of translation	0.00082	191	15
GO:0090382	phagosome maturation	0.00087	50	8
GO:0030433	ubiquitin-dependent ERAD pathway	0.00093	115	13
GO:0031145	anaphase-promoting complex-dependent catabolic process	0.00095	172	24

GO:0010940	positive regulation of necrotic cell death	0.00098	28	5
GO:0040027	negative regulation of vulval development	0.00100	137	17
GO:0006626	protein targeting to mitochondrion	0.00103	92	14
GO:0044281	small molecule metabolic process	0.00108	760	47
GO:0035459	cargo loading into vesicle	0.00108	23	4
GO:0045116	protein neddylation	0.00117	28	7
GO:0050830	defense response to Gram-positive bacterium	0.00118	80	10
GO:0060212	negative regulation of nuclear-transcribed mRNA poly(A) tail shortening	0.00125	24	6
GO:0060315	negative regulation of ryanodine-sensitive calcium-release channel activity	0.00135	32	7
GO:0051701	interaction with host	0.00136	51	8
GO:0002479	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	0.00138	137	20
GO:0009965	leaf morphogenesis	0.00139	32	7
GO:0000387	spliceosomal snRNP assembly	0.00140	51	11
GO:0061077	chaperone-mediated protein folding	0.00166	86	11
GO:0045446	endothelial cell differentiation	0.00166	34	5
GO:0009411	response to UV	0.00172	95	9
GO:0006997	nucleus organization	0.00172	66	9
GO:0042981	regulation of apoptotic process	0.00182	245	14
GO:0042921	glucocorticoid receptor signaling pathway	0.00184	41	5
GO:0033209	tumor necrosis factor-mediated signaling pathway	0.00185	179	21
GO:0016036	cellular response to phosphate starvation	0.00188	20	4
GO:0022008	neurogenesis	0.00191	291	25
GO:0000395	mRNA 5'-splice site recognition	0.00203	26	6
GO:0006879	cellular iron ion homeostasis	0.00220	134	14
GO:0033120	positive regulation of RNA splicing	0.00223	53	6
GO:0048680	positive regulation of axon regeneration	0.00226	23	5
GO:0006091	generation of precursor metabolites and energy	0.00234	41	7
GO:0007140	male meiotic nuclear division	0.00236	54	7
GO:0006337	nucleosome disassembly	0.00242	51	5
GO:0022417	protein maturation by protein folding	0.00269	31	6
GO:0030836	positive regulation of actin filament depolymerization	0.00271	22	5
GO:0097167	circadian regulation of translation	0.00280	24	4

GO:0030042	actin filament depolymerization	0.00287	26	4
GO:0050832	defense response to fungus	0.00290	83	8
GO:0035067	negative regulation of histone acetylation	0.00290	22	4
GO:0071901	negative regulation of protein serine/threonine kinase activity	0.00298	46	5
GO:0006368	transcription elongation from RNA polymerase II promoter	0.00315	131	14
GO:0009793	embryo development ending in seed dormancy	0.00326	99	12
GO:0045727	positive regulation of translation	0.00329	230	17
GO:0035071	salivary gland cell autophagic cell death	0.00335	58	10
GO:0010467	gene expression	0.00347	292	22
GO:0021762	substantia nigra development	0.00349	174	17
GO:0006605	protein targeting	0.00399	124	9
GO:0044723	NA	0.00420	31	5
GO:0007095	mitotic G2 DNA damage checkpoint	0.00431	130	12
GO:0000226	microtubule cytoskeleton organization	0.00437	174	11
GO:0007280	pole cell migration	0.00451	23	3
GO:0006334	nucleosome assembly	0.00451	90	9
GO:0006336	DNA replication-independent nucleosome assembly	0.00465	26	5
GO:0000245	spliceosomal complex assembly	0.00476	81	11
GO:0048205	COPI coating of Golgi vesicle	0.00497	38	6
GO:0051436	negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	0.00500	113	17
GO:0002576	platelet degranulation	0.00510	137	12
GO:0050434	positive regulation of viral transcription	0.00548	77	10
GO:0061158	3'-UTR-mediated mRNA destabilization	0.00559	28	4
GO:0097193	intrinsic apoptotic signaling pathway	0.00562	128	11
GO:0070124	mitochondrial translational initiation	0.00581	114	18
GO:0032940	secretion by cell	0.00588	90	10
GO:0000165	MAPK cascade	0.00597	386	23
GO:0043162	ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway	0.00612	58	7
GO:0035329	hippo signaling	0.00612	61	5
GO:0007281	germ cell development	0.00620	164	13
GO:0071392	cellular response to estradiol stimulus	0.00631	105	8

GO:0030431	sleep	0.00647	75	7
GO:0070126	mitochondrial translational termination	0.00656	116	18
GO:0016236	macroautophagy	0.00661	72	8
GO:0000183	chromatin silencing at rDNA	0.00698	37	5
GO:0009408	response to heat	0.00715	212	16
GO:0008103	oocyte microtubule cytoskeleton polarization	0.00723	44	5
GO:0030182	neuron differentiation	0.00737	152	11
GO:0044804	autophagy of nucleus	0.00750	35	6
GO:0030866	cortical actin cytoskeleton organization	0.00791	105	7
GO:0021549	cerebellum development	0.00793	178	11
GO:0007265	Ras protein signal transduction	0.00853	460	27
GO:0007291	sperm individualization	0.00923	46	6
GO:0000082	G1/S transition of mitotic cell cycle	0.00929	360	27
GO:0007088	regulation of mitotic nuclear division	0.00932	89	7
GO:0042692	muscle cell differentiation	0.00986	75	6
GO:0010388	NA	0.01007	39	7
GO:0006977	DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	0.01010	149	18
GO:0033572	transferrin transport	0.01014	86	8
GO:0000090	mitotic anaphase	0.01057	327	27
GO:0043388	positive regulation of DNA binding	0.01090	90	9
GO:0010228	vegetative to reproductive phase transition of meristem	0.01092	30	5
GO:0038095	Fc-epsilon receptor signaling pathway	0.01092	595	31
GO:1901800	positive regulation of proteasomal protein catabolic process	0.01105	41	5
GO:0048190	wing disc dorsal/ventral pattern formation	0.01146	31	3
GO:0070125	mitochondrial translational elongation	0.01152	120	18
GO:0046716	muscle cell cellular homeostasis	0.01183	79	7
GO:0006376	mRNA splice site selection	0.01186	58	7
GO:0071230	cellular response to amino acid stimulus	0.01209	130	8
GO:0045792	negative regulation of cell size	0.01212	52	5
GO:0048013	ephrin receptor signaling pathway	0.01246	316	15
GO:0000003	reproduction	0.01273	541	39
GO:0051683	establishment of Golgi localization	0.01296	39	5

GO:0007019	microtubule depolymerization	0.01338	31	5
GO:0061484	hematopoietic stem cell homeostasis	0.01344	26	4
GO:0021766	hippocampus development	0.01350	231	10
GO:1901224	positive regulation of NIK/NF-kappaB signaling	0.01354	31	3
GO:0008039	synaptic target recognition	0.01371	21	4
GO:0071732	cellular response to nitric oxide	0.01393	43	4
GO:0006623	protein targeting to vacuole	0.01412	54	7
GO:0007422	peripheral nervous system development	0.01427	151	9
GO:0086013	membrane repolarization during cardiac muscle cell action potential	0.01464	31	3
GO:1901016	regulation of potassium ion transmembrane transporter activity	0.01490	26	3
GO:0042787	protein ubiquitination involved in ubiquitin-dependent protein catabolic process	0.01497	216	13
GO:0018996	molting cycle, collagen and cuticulin-based cuticle	0.01513	127	13
GO:0034660	ncRNA metabolic process	0.01548	42	8
GO:0000045	autophagosome assembly	0.01552	87	8
GO:0043206	NA	0.01648	23	3
GO:0010881	regulation of cardiac muscle contraction by regulation of the release of sequestered calcium ion	0.01659	78	6
GO:0009737	response to abscisic acid	0.01679	49	6
GO:0090090	negative regulation of canonical Wnt signaling pathway	0.01680	396	22
GO:0032516	positive regulation of phosphoprotein phosphatase activity	0.01699	38	4
GO:0010976	positive regulation of neuron projection development	0.01708	356	16
GO:0045087	innate immune response	0.01709	1119	51
GO:0043087	regulation of GTPase activity	0.01748	101	6
GO:0006509	membrane protein ectodomain proteolysis	0.01757	53	5
GO:0090307	mitotic spindle assembly	0.01759	113	10
GO:2001243	negative regulation of intrinsic apoptotic signaling pathway	0.01795	60	6
GO:0060306	regulation of membrane repolarization	0.01800	23	3
GO:0033523	histone H2B ubiquitination	0.01805	29	5
GO:0055007	cardiac muscle cell differentiation	0.01816	75	4
GO:0045132	meiotic chromosome segregation	0.01858	45	5
GO:0034047	NA	0.01900	44	5
GO:0072499	photoreceptor cell axon guidance	0.01910	25	3
GO:0072593	reactive oxygen species metabolic process	0.01926	69	7

GO:0034454	microtubule anchoring at centrosome	0.01934	20	4
GO:0006661	phosphatidylinositol biosynthetic process	0.01956	112	7
GO:0036099	female germ-line stem cell population maintenance	0.01979	35	6
GO:0051489	regulation of filopodium assembly	0.01990	73	6
GO:0016239	positive regulation of macroautophagy	0.01998	35	3
GO:0016568	NA	0.02007	126	8
GO:0010390	histone monoubiquitination	0.02044	44	5
GO:0044458	motile cilium assembly	0.02053	29	4
GO:0010459	negative regulation of heart rate	0.02073	29	4
GO:0048812	neuron projection morphogenesis	0.02126	162	9
GO:0043281	regulation of cysteine-type endopeptidase activity involved in apoptotic process	0.02128	64	4
GO:0045292	mRNA cis splicing, via spliceosome	0.02128	28	5
GO:0007183	SMAD protein complex assembly	0.02156	23	3
GO:0019886	antigen processing and presentation of exogenous peptide antigen via MHC class II	0.02222	208	11
GO:0031987	locomotion involved in locomotory behavior	0.02227	36	3
GO:0009853	photorespiration	0.02228	45	7
GO:0048387	negative regulation of retinoic acid receptor signaling pathway	0.02258	30	3
GO:0043652	engulfment of apoptotic cell	0.02282	51	6
GO:0006465	signal peptide processing	0.02315	24	4
GO:0000132	establishment of mitotic spindle orientation	0.02320	101	7
GO:0006412	translation	0.02371	151	13
GO:0048477	oogenesis	0.02387	187	13
GO:0007288	sperm axoneme assembly	0.02545	26	3
GO:0030177	positive regulation of Wnt signaling pathway	0.02552	147	7
GO:0043488	regulation of mRNA stability	0.02553	47	5
GO:0044708	NA	0.02586	32	3
GO:0007369	gastrulation	0.02593	79	7
GO:0042048	olfactory behavior	0.02598	52	3
GO:0000084	mitotic S phase	0.02608	200	19
GO:1900028	negative regulation of ruffle assembly	0.02781	26	3
GO:1901841	regulation of high voltage-gated calcium channel activity	0.02790	30	4
GO:0001731	formation of translation preinitiation complex	0.02807	45	7

GO:0007179	transforming growth factor beta receptor signaling pathway	0.02835	374	18
GO:0006417	regulation of translation	0.02845	123	10
GO:0046329	negative regulation of JNK cascade	0.02944	98	5
GO:0009407	toxin catabolic process	0.02950	32	6
GO:0035046	pronuclear migration	0.02964	51	6
GO:0031000	response to caffeine	0.02984	40	4
GO:0006783	heme biosynthetic process	0.03021	31	5
GO:0002322	B cell proliferation involved in immune response	0.03035	22	3
GO:0061179	negative regulation of insulin secretion involved in cellular response to glucose stimulus	0.03100	35	4
GO:1901673	regulation of mitotic spindle assembly	0.03106	28	4
GO:0042098	T cell proliferation	0.03212	54	5
GO:0043951	negative regulation of cAMP-mediated signaling	0.03217	38	4
GO:0006734	NADH metabolic process	0.03245	26	4
GO:2000300	regulation of synaptic vesicle exocytosis	0.03245	25	3
GO:0002168	instar larval development	0.03279	24	3
GO:0051280	negative regulation of release of sequestered calcium ion into cytosol	0.03292	21	3
GO:0051775	response to redox state	0.03326	34	4
GO:0002407	dendritic cell chemotaxis	0.03338	22	3
GO:0008582	regulation of synaptic growth at neuromuscular junction	0.03376	39	4
GO:0030307	positive regulation of cell growth	0.03471	225	13
GO:0000724	double-strand break repair via homologous recombination	0.03500	88	8
GO:0032410	negative regulation of transporter activity	0.03565	23	3
GO:0006344	maintenance of chromatin silencing	0.03584	24	3
GO:0033554	cellular response to stress	0.03818	205	12
GO:0033227	dsRNA transport	0.03860	38	4
GO:0000186	activation of MAPKK activity	0.03932	452	24
GO:0031115	negative regulation of microtubule polymerization	0.03944	59	5
GO:0045947	negative regulation of translational initiation	0.03946	44	4
GO:0042407	cristae formation	0.03966	27	4
GO:0010172	embryonic body morphogenesis	0.03984	55	4
GO:0043044	ATP-dependent chromatin remodeling	0.04026	86	5
GO:0042326	negative regulation of phosphorylation	0.04093	38	4

GO:0016477	cell migration	0.04093	491	19
GO:0032007	negative regulation of TOR signaling	0.04105	77	5
GO:0010633	negative regulation of epithelial cell migration	0.04112	63	4
GO:0035584	calcium-mediated signaling using intracellular calcium source	0.04142	65	4
GO:0007346	regulation of mitotic cell cycle	0.04173	132	7
GO:0008625	extrinsic apoptotic signaling pathway via death domain receptors	0.04241	76	5
GO:0008407	chaeta morphogenesis	0.04248	36	4
GO:0048010	vascular endothelial growth factor receptor signaling pathway	0.04316	610	28
GO:0051224	negative regulation of protein transport	0.04331	24	3
GO:0006913	nucleocytoplasmic transport	0.04353	151	10
GO:0048793	pronephros development	0.04368	75	6
GO:0045165	cell fate commitment	0.04450	75	4
GO:2000778	positive regulation of interleukin-6 secretion	0.04456	26	4
GO:0001649	osteoblast differentiation	0.04471	257	16
GO:0032008	positive regulation of TOR signaling	0.04500	48	5
GO:0017145	stem cell division	0.04546	21	2
GO:0043123	positive regulation of I-kappaB kinase/NF-kappaB signaling	0.04574	298	17
GO:0008156	negative regulation of DNA replication	0.04690	37	4
GO:0031398	positive regulation of protein ubiquitination	0.04733	119	7
GO:0043983	histone H4-K12 acetylation	0.04735	33	3
GO:0043537	negative regulation of blood vessel endothelial cell migration	0.04819	33	4
GO:0006939	smooth muscle contraction	0.04858	50	3

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