The influence of structural complexity on phenotypic development and post-release performance of juvenile Atlantic salmon (*Salmo salar*)

By

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Abstract

The environment can play an integral role on phenotypic development of an organism. In this thesis, I experimentally tested the influence of structural complexity on phenotypic development and post-release performance of juvenile Atlantic salmon (*Salmo salar*) when released into semi-natural and natural environments. From an applied perspective, I was interested in investigating alternative rearing strategies of juvenile *Salmo salar* with the goal of enhancing performance of fish when released into nature.

In my first data chapter, I conducted an experiment to test the influence of environmental enrichment during incubation (i.e. addition of gravel to create a “complex” environment) on the phenotype and performance of juvenile salmon in a semi-natural environment. At the culmination of endogenous feeding (i.e. “emergence”), fish that incubated in a complex environment were heavier, in better body condition, fed more on novel prey, and took longer to reappear from a shelter after a simulated predator attack. In addition, when transferred from incubation environments to semi-natural stream channels, fish originating from the complex incubation environment expressed enhanced growth and survival. These findings suggest that *Salmo salar* are plastic to environmental factors during incubation and developmental differences could be contributing to performance in semi-natural stream channels. There were, however, no commensurate differences in brain volume with those observed in behaviour and performance, which may suggest that other developmental processes in the brain of juvenile Atlantic salmon are occurring that are not reflected in overall brain volume.

In my second data chapter, I reared the fish from the preceding incubation study for 60 days after emergence in tanks with ("complex") or without cobble substrate ("simple"). I used a reciprocal approach to rearing and moved fish between complex and simple environments for two 30-day rearing periods to investigate if timing or duration of timing of exposure to habitat complexity influenced growth and condition in the hatchery and subsequent survival in semi-natural and wild environments. I found that fish reared in complex tanks exhibited higher body condition (i.e. heavier for a given length) for the first 30 days, however, differences generally faded during the second 30 days of hatchery rearing. Additionally, I observed rearing groups with smaller fish, on average, at release expressed higher growth rates in semi-natural stream channels and in the wild, such that sizes and conditions did not differ among groups at final recapture. Estimated survival after release into the wild (over ca. 260 days) was higher for fish incubated in a complex environment and reared in a simple environment for consecutive 30 d rearing periods (Complex>Simple>Simple, or "CSS") and those in SCC treatment than fish from 3 of the 7 other groups (CCC, CSC, SSC). There were no other differences in survival between treatment groups. Survival in the semi-natural stream channels was high (> 85%) and I did not detect differences between groups.

The results presented in this thesis suggest that phenotypic traits of juvenile Atlantic salmon are sensitive to environmental factors early in development. Enriched environments containing gravel and cobble may serve to provide cover for juvenile fish during early life, allowing them to rest, hide from predators, metabolize, and allocate energy towards somatic growth and development. Such enrichment can provide fish an advantage when released into the wild, though effects may be complicated. As a rearing

strategy for the release of salmon into nature to re-establish depleted populations, modifications are likely needed to simplify maintenance of rearing tanks, however this study serves as a benchmark for future efforts at alternative rearing techniques for enhancing performance of released juvenile *Salmo salar* in nature.

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Chapter 1. Introduction

Phenotypic traits are expressed in response to the interaction of an organism's genetic makeup and the environment it experiences throughout development. Thus, the environment, which is variable over an organism's life cycle, can influence the way in which a particular trait is expressed and this response is often referred to as phenotypic plasticity (West-Eberhard 2003, Hutchings 2011). Whether these responses are in terms of physiology, behaviour, or morphology, the ability to adapt to the environment via phenotypic plasticity has direct consequences on the fitness of an organism.

Many species of animals are reared in captivity for a number of reasons, including release into nature for conservation purposes or to supplement harvesting opportunities. Captive environments are generally simple by nature and lack many of the influences that an animal may experience in the wild. Thus, captive rearing environments can play a large role on phenotypic development, including neural development, behaviour, and morphology, in a wide range of species (Fish: Fleming and Einum 1997, Kihslinger and Nevitt 2006, Dhanasiri et al. 2011, De Mestral et al. 2013, Birds: Ewenson et al. 2001, Guay et al. 2008, Mammals: Maki et al. 1993, Hunter et al. 2002, O'Regan and Kitchener 2005). Rearing animals for release into nature can thus pose a significant problem since traits that are often selected against in nature can persist in captive environments. In other words, captive-reared animals may be at a disadvantage when released into wild environments.

Salmonid fish are commonly reared in captive environments for fisheries supplementation and biodiversity conservation. However, restocking efforts are often inhibited by poor survival of captive-reared fish in nature. In light of this situation,

salmon provide a wealth of opportunity to study environmental influences on phenotypic development. Fish of the Salmonidae family exhibit complex life cycles in which they must cope and adapt to a wide range of environmental conditions. Furthermore, salmon have been exploited for centuries, populations are declining, and restocking efforts often struggle, as captive-reared fish tend to perform poorly in wild environments (Jonsson and Jonsson 2006). Therefore, research is needed to mitigate the issue of poor survival of captive-reared fish in the wild.

Atlantic salmon

Atlantic salmon are distributed throughout coastal rivers of the North Atlantic but many populations are declining (Parrish et al. 1998). Atlantic salmon most commonly exhibit an anadromous life history, i.e. they migrate to sea for the majority of their adult life stage and rear in freshwater as juveniles. However, there are populations that spend their entire life cycle in fresh water (Jonsson and Jonsson 2011).

Atlantic salmon can spend 1-4 years in the sea before migrating back to their natal river to spawn (which tends to occur in Autumn). Once females move upstream and find a proper nesting site, they will dig nests in the gravel in a location (i.e. a redd) where water flow constantly provides deposited eggs with oxygenated water and impedes sediment accumulation (most commonly in riffle areas). Eggs remain in the shelter of the gravel nest for up to 4 months where they will incubate, hatch as 'alevins' (larvae) and persist off of endogenous energy reserves (yolk sac) that remain from the egg. Once this internal energy source is depleted, the young salmon (fry) must emerge from the gravel nest and begin exogenous feeding in the stream environment where they experience intense competition and predation pressure. This stage of early ontogeny, often referred to as

‘emergence,’ is labeled as a critical period in which high levels of mortality are experienced due to predation and starvation (Fleming and Einum 2011). Fish will continue to rear in freshwater habitats for 1-5 years, depending on environmental conditions, until they undergo significant changes in behaviour, morphology, and physiology in preparation for their seaward migration (i.e. smolting) (McCormick et al. 1987).

Captive rearing of salmon

For restocking purposes, salmon are often reared in controlled facilities and released into nature at a certain developmental stage in early ontogeny, depending on the management strategy. Fish rearing facilities are strikingly different than wild environments in that they generally consist of barren rearing tanks of which fish are fed nutritional pellets to satiation and are not exposed to predators. Therefore, selection forces in captivity favour traits most appropriate for such environments, for example aggressive behaviour and rapid growth (Fleming and Einum 1997, Huntingford 2004, Sundström et al. 2004, Jonsson and Jonsson 2006). Thus, it has been observed that captive-reared fish exhibit phenotypic divergence from their wild counterparts and often perform poorly when they are released into nature (Fleming and Einum 1997, Von Cramon-Taubadel et al. 2005, Jonsson and Jonsson 2006, Araki et al. 2007, Belk et al. 2008).

A recent movement focusing on better preparing captive-reared organisms for life in the wild is taking form. This movement is attempting to identify rearing techniques that will promote the expression of phenotypic traits that are more favourable for life in the wild. For example, investigations for whooping crane and other bird species re-introduction have identified early-life rearing techniques to promote behaviours that could

increase survival in the wild (Van Heezik et al. 1999, Kreger et al. 2006). Currently, a large body of research investigating alterations in captive fish rearing facilities to enhance post-release survival is ongoing. Alterations in rearing environments such as adding habitat complexity to early rearing environments, decreasing fish density, and altering food quality and abundance are a few manipulations that have drawn attention. It has been suggested that a lack of structural complexity in captive fish rearing environments limits opportunities for learning adaptive behaviours (Brown and Laland 2001). It is not surprising therefore, that adding structural complexity to rearing environments has resulted in stimulated neural development (Kihslinger and Nevitt 2006, Salvanes et al. 2013), enhanced foraging abilities (Brown et al. 2003, Rodewald et al. 2011), and reduced risk-taking behaviour (Roberts et al. 2011) of juvenile Atlantic salmon (*Salmo salar*) and also promoted behavioural flexibility in Atlantic cod (*Gadus morhua*) (Braithwaite and Salvanes 2005). This evidence gives support to the theory that adding structural complexity to captive rearing environments could facilitate the expression of phenotypic traits that could be advantageous in nature.

This Thesis

In this thesis, I attempted to better understand the influence of structural complexity during early ontogeny on phenotypic development and performance of juvenile Atlantic salmon (*Salmo salar*) and how this influence, if any, could enhance post-release performance for restocking purposes. In Chapter Two, I undertook an investigation on the influence of incubation environment on phenotypic traits (body size, brain volume, feeding behaviour) and subsequent performance in a semi-natural environment. I aimed to test the following predictions:

Increased structural complexity can benefit salmon by providing cover for resting and metabolizing, and spatial landmarks for neural stimulation and development. Such environmental characteristics could 1) allow for increased endogenous resource allocation towards somatic growth and neural development (i.e. larger fish and larger brains), 2) facilitate the ability to develop adaptive behaviour (e.g. seeking shelter or feeding on novel/live prey), and 3) subsequently enhance post-release performance.

In Chapter Three, I reared fish in structurally complex environments beyond the endogenous feeding stage of incubation. I employed a 'reciprocal' rearing design of which fish from the two incubation environments (simple and structurally complex) in Chapter Two were split into rearing tanks with and without structural complexity (i.e. normal hatchery conditions). Fish reared in these tanks for 30 days at which point they were switched into tanks with or without structural complexity again for another 30 days. At the end of the captive rearing period, I marked fish with individual tags and released them into a nearby stream and a semi-natural stream at the hatchery to assess post-release performance (in terms of survival and growth). With this design, I hoped to identify if timing or duration of timing of exposure to habitat complexity would influence post-release performance. From a management perspective, this design could be important in identifying more labour and cost effective methods of rearing fish for enhancing post-release performance. In this chapter, I aimed to test the following prediction:

Increased exposure (timing) to structural complexity during captive rearing will increase post-release performance in terms of growth and survival.

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Authorship statement

I wrote the general introduction and conclusion with comments from my advisor, Dr. Ian Fleming. The two data chapters (2 & 3) were co-written with my advisor, Dr. Ian Fleming.

Chapter 2. Structural complexity during incubation influences behaviour and performance but not brain size of emergent Atlantic salmon (*Salmo salar*)

Abstract
The environment experienced early in ontogeny can greatly influence phenotypic development and fitness of an organism. Salmon are reared in captive environments for many reasons, including restocking into nature. Phenotypic traits of salmon reared in captivity are markedly different than those of their wild counterparts and it has been observed that captive-reared fish typically perform poorly in wild environments. Recent efforts have attempted to mitigate this problem by manipulating conditions in fish-rearing facilities to promote the expression of phenotypic traits that may be more favourable in nature. In this study, we incubated Atlantic salmon (*Salmo salar)* eggs in two environments (with and without gravel) until emergence ('swim-up') and measured brain volume, body size and condition, feeding behaviour, response to a simulated predator, and performance in semi-natural stream channels. We found that gravel-incubated fish were heavier and in better condition, fed more readily on live prey, and outperformed (in terms of growth and survival) non gravel-incubated fish in semi-natural stream channels. In addition, fish from the complex incubation environment took on average longer to reappear from shelter after a simulated predator attack. We did not detect differences (absolute or size-corrected) in whole brain, telencephalon, or olfactory bulb volumes of fish incubated in the two environments. Our results suggest that adding gravel to incubation environments in captivity can have a significant influence on phenotypic development of juvenile Atlantic salmon and that gravel-incubated salmon may have an advantage if releasing them into the wild for restocking.

Introduction

Phenotypic traits are expressed in response to an organism's genetic makeup and the environment it experiences throughout development. Early life experiences can significantly influence phenotypic development of an organism both immediately and later in life (Metcalfe and Monaghan 2001, West-Eberhard 2003, Wu et al. 2012). It is not surprising, therefore, that raising animals in captivity can have profound effects on phenotypic development (Kostkow 2004, Connolly and Cree 2008, Portugal et al. 2011).

Captive environments are generally simple by nature and lack the stimulation that an animal may experience in the wild. Subsequently, effects on morphology, behaviour, and physiology in response to captive-rearing conditions have been observed in a plethora of species (Maki et al. 1993, Fleming and Einum 1997, Wiedenmayer 2009, Brzek et al. 2011, Dhanasiri et al. 2011, Wang et al. 2011, De Mestral et al. 2013). For example, rearing in captivity has been observed to negatively influence neural development in a variety of species (Kruska 1980, Kihslinger and Nevitt 2006, Ali et al. 2009, Chen and Chen 2012, Guay et al. 2012, Näslund et al. 2012). Recently, a growing trend of studies investigating brain development in captive reared fish has emerged (Kihslinger et al. 2006a, b, Burns et al. 2009, Kotrshcal et al. 2012, Näslund et al. 2012, Salvanes et al. 2013). These issues have fueled concerns regarding the welfare of animals reared in captivity and the consequences that such influences could pose to fitness if animals are being raised for reintroduction into nature.

Captive rearing of fish to supplement declining wild populations is a common practice in many countries. However, fish reared in captive environments have been observed to develop maladaptive fitness-related traits (Einum and Fleming 2001,

Marchetti and Nevitt 2003, Kihslinger et al. 2006, Salvanes and Braithwaite 2006, Näslund et al. 2012). More specifically, rearing in captive environments can inhibit foraging abilities and predator avoidance and enhance aggression in juvenile salmon and other fish species (Brown et al. 2003, Braithwatie and Salvanes 2005, Lee and Berejikian 2008, Tatara et al. 2008). This is not surprising as fish reared in captivity are typically fed pellets to satiation, never exposed to predation pressure, and reared in tanks at high densities that are void of habitat complexity. Therefore, it is likely that poor performance observed in captive fish in nature support the hypothesis that captive environments do not adequately prepare fish for survival in the wild (Maynard et al. 1995, Le Vay et al. 2007, Jonsson and Jonsson 2009, Thorstad et al. 2011).

Neurogenesis occurs in the fish brain throughout its lifetime and this leaves potential for plastic responses to environmental factors that could stimulate neural development (Zupanc et al. 2005). Some recent studies have reported smaller whole brain or brain sub region sizes in fish reared in ‘barren’ or captive environments compared to fish reared in ‘enriched’ (e.g. modifications in captivity) or wild environments (Marchetti and Nevitt 2003, Kihslinger et al. 2006, 2006, Näslund et al. 2012). However, this trend may not be very general, as the opposite pattern of larger brains in captive reared fish has recently been observed (Kotrshcal et al. 2012). Whereas captive-reared fish are said to have lower fitness levels than wild fish in nature (e.g. Araki et al. 2007), mixed observations of brain size, as noted above, lead to speculation regarding the assumption of overall brain size as an indicator of cognitive ability (Healy and Rowe 2007), Thus, more studies are needed to understand the relationship between fitness and brain size in juvenile salmon.

In this experimental study, we investigated the influence of incubation environment on phenotypic traits of young-of-the-year Atlantic salmon (*Salmo salar*). More specifically, we aimed to understand how habitat complexity during incubation influences morphology (body and brain size), behaviour, and performance in a semi-natural environment of juvenile salmon. We tested the predictions that incubating in a more 'natural' environment allows for more efficient endogenous energy allocation towards somatic and neural growth resulting in larger fish with larger brains (see Millidine et al. 2006). Furthermore, we hypothesized that influences on morphology and neural development would facilitate the development of adaptive behaviour (feeding on novel / live prey and response to a simulated predator) and increase performance in a semi-natural environment.

Methods

Experimental Fish

Gametes (full siblings) were collected from 85 female and 85 male Atlantic salmon (*Salmo salar*) from the Tobique River, NB (46.8°N, 67.8°W) in November 2011. These adults, captured in the Tobique River during their seaward migration (i.e. as smolts), had been reared for 1.5 years to maturity at the Mactaquac Biodiversity Centre (MBC), Department of Fisheries and Oceans, New Brunswick, Canada, as part of a salmon enhancement and conservation program. Fertilized eggs were held in a common environment (egg trough) until they began to develop visible eyes at which point they could be handled without mortality. Then 4,000 eggs were haphazardly assigned to each of eight experimental incubation units on 18 February 2011, where they reared until

emergence (i.e. the start of exogenous feeding). By haphazardly distributing the fertilized eggs from the common pool of crosses we aimed to minimize genetic and other parental effects (Kennedy et al. 2008).

Experimental Design

Incubation units (108 x 55.2 x 44.5 cm) consisted of four replicates of each of two environments (simple and complex) and were positioned in an alternating fashion over a conventional egg trough (Fig. 1). Ambient Saint John River water flowed from one main pipe at the head of the trough and split into each side of the trough and into each incubation unit at 0.37 $L \bullet s^{-1}$. Each incubation unit had an adjustable valve, which allowed us to make flows consistent across replicates. Water entered through a chamber beneath the egg incubation area and percolated upwards and out through two outflow pipes at the top of each incubation unit (Fig. 2), which were covered with a fine mesh screen in simple treatments to prevent escape. The four 'complex' incubation units were filled with 10 cm of gravel (ca. 3.8 mm in diameter), 5 cm of semi-hollow plastic rings (5 x 2.5 cm) as an incubation medium where eggs were carefully placed (to ensure eggs were not damaged), and topped with an additional 10cm of gravel (Fig. 2). Gravel used in the incubation units came from a quarry and as such, had not been exposed recently to aquatic organisms. The four 'simple' incubation units were identical to the complex incubation units except they contained no substrate of any kind and had mesh screens on outflow pipes to prevent escape. All units were set up and fully functioning for 7 days prior to eyed egg introduction. Due to a malfunction in the regulation of the temperature in one of the complex incubation units, it was excluded from analyses.

During emergence, the juveniles (i.e. fry) work their way through the gravel in preparation to begin exogenous feeding. Outflow pipes on the complex incubation units emptied into emergent traps to allow for the monitoring of fry emergence. The traps were checked daily and the fry returned to the incubation unit. Simple incubation units were checked at the same time as complex units in an attempt to ensure a similar "disturbance" regime across treatments.

Sampling

Sampling occurred at emergence, which was defined as the date when at least 500 fish were found in each of the complex incubation unit emergence traps. This occurred on 15 June 2012. At that point, 30 fish were haphazardly netted from each complex incubation unit emergent trap and from inside each simple incubation unit. Fish were euthanized by an over-dose of MS222, photographed, weighed to the nearest 0.001 g, measured for fork length to the nearest 0.1 cm, and preserved immediately in 4 % paraformaldehyde for subsequent neural analysis. After 24 h in paraformaldehyde, the preserved fry were transferred to 95% ethanol for storage (Kihslinger and Nevitt 2006, Näslund et al. 2012).

Neural analysis

Heads of preserved fish were carefully dissected from bodies directly posterior of the operculum. Brains were not removed from heads because of the small size of the fish and the potential to damage tissues. Instead whole heads were embedded in paraffin using an automated tissue processor, TissueTek VIP 6. Paraffin blocks were cut sagittally on a Leica RM2125RT microtome at a thickness of 14 µm. Glass slides were prepared with 10 sections per slide and heated at 60°C for 2 h. The slides were stained using Hematoxylin

and Eosin methods (Mizoguchi and Kikui 1987, Appendix A). Cross-sectional area of the total brain (and identified structures, olfactory bulb and telencephalon) were measured serially and analyzed at regular 32 µm intervals using ImageJ 1.45s software (Fig. 3). The person measuring the area was blind to treatment. Volume of the whole brain and measured substructures (telencephalon and olfactory bulbs) were calculated by multiplying the area of each slice by its thickness and then summing across slices. Measurements of the whole brain were taken from when the first cells of the optic tectum were observed in the slice sequence.

Behaviour experiments

From 2-8 July 2011, experiments on the reaction of newly emerged Atlantic salmon from the two incubation environments to novel, live prey was conducted in 15 experimental arenas (18 x 11.5 x 5 cm, Fig. 4). Fish were transitioning from endogenous to exogenous feeding and were unfed prior to the experiment. Each arena contained one plastic hide (4 x 4 cm) at the downstream end. A total of 76 fish were tested for foraging behaviour (n=38 complex, n=38 simple spread across different incubation units). Individual fish were placed into arenas the evening prior to observation, which took place the next morning (allowing at least 16 h acclimation). At the onset of each observation, 5 ml of live *Artemia* was injected into the upstream end of an individual observation arena from behind a blind. Simultaneous to prey injection, a stopwatch was started and the fish being tested was observed for a total of 90 s. Fish were assigned a score of “fed” or “did not feed” depending on its reaction to *Artemia* within the 90-s observation period. Stomach contents were not analyzed and thus, feeding success was not measured. Fish

that began in the hide at the onset of the experiment were excluded. To control for arena effects, fish from each treatment were tested in each experimental arena.

Experiments on the reaction of newly emerged Atlantic salmon (unfed) from the two incubation environments to a simulated predator was conducted in the same arenas as used in the feeding experiment during 22 June - 8 July 2011. A total of 68 fish were tested from the two incubation environments (n = 30 complex and n = 38 simple). Fish were placed into arenas at least 16 h prior to observations. At the onset of each observation, a video recording was started and a simulated blue heron model was inserted into the water in the middle of the observation arena from behind a blind. The model heron was moved in a circular manner for 5 s and removed. Fish were assigned a score of "reacted" or "did not react," "found shelter" or "did not find shelter," and if a fish found the shelter the time elapsed from when the fish entered the shelter to when it reappeared was noted. A "reaction" constituted a fish moving from one location to another during or immediately following simulated attack. Fish were recorded for a maximum of 15 minutes. Fish that began in the hide at the onset of the experiment were excluded. To control for arena effects, fish from each treatment were tested in each experimental arena.

Performance in semi-natural stream channels

To assess growth and survival of fish from the two incubation environments under semi-natural conditions, an experiment was conducted using eight outdoor stream channels (Fig. 5). Each streambed was covered with natural gravel substrate, water velocities ranged from 1 to 4 cm•s^{-1} and depths were between 0.23 m and 0.27 m. Water from the Saint John River flowed from one main pipe and was split into 8 stream channels with individually controlled valves. Flow was initiated in each stream 21 d prior

to the experiment to allow for natural colonization of invertebrates to provide a diverse prey fauna for juvenile fish. Only natural prey was available for fish throughout the course of the experiment and mean water temperature was 15.2°C, ranging from 14°C to 16°C.

The experiment was conducted between 28 June and 9 August (42 d). Fish were in the emergent stage and were unfed prior to introduction to streams. Eight groups of 80 juveniles, consisting of 40 from complex incubation units and 40 from simple incubation units, were haphazardly chosen, with representation from across the various incubation units, measured (fork length and weight) and marked to incubation treatment with visual implanted elastomer (VIE) tags implanted at the base of the dorsal fin (Fig. 6). Densities were chosen to encourage fish to compete for resources within the stream channels. To ensure fish were healthy after tagging, each group was held separately for at least 24 h in 1-m circular tanks before being released into the eight stream channels.

At the end of the experiments, a modified seine net was used to herd fish downstream where they were netted. Each stream then was drained to ensure that all fish were collected. Surviving fish were identified and measured (fork length and weight).

Statistical analysis

Treatment effects on body size (log mass [M] or log length [L]) at emergence were analyzed using a two-way nested ANOVA model where replicate tank (random factor) was nested within Treatment (fixed factor). To examine body condition, residuals were produced from a regression analysis of log L (x-axis) and log M (y-axis) measurements

and used as a response variable in a two-way nested ANOVA model where replicate tank (random factor) was nested within Treatment (fixed factor).

Absolute volumes of the whole brain and measured sub regions (telencephalon and olfactory bulbs) were log transformed and compared among treatments using a two-way nested ANOVA model where replicate tank (random factor) was nested within Treatment (fixed factor). To control for body size effects, whole brain and brain region volumes were also analyzed using analysis of covariance (ANCOVA) where fork length and weight were used as covariates in separate ANCOVA models. Fork lengths used in ANCOVA models were taken from images of the individual fish using ImageJ 1.45s software for more accurate length measurements. The replicate tank effect was removed from final ANCOVA models because it was non-significant ($p > 0.05$).

Individual feeding behaviour of fish from the two incubation environments was analyzed by assigning each fish a binomial score “fed” or “did not feed.” The score was given to each individual fish depending on whether it fed or not during the 90-s observation period. We used a generalized linear mixed model with feeding reaction as a response variable, treatment as a fixed factor, arena as a random factor, and a logit link function for data with a binomial distribution. Arena effect was non-significant and removed from the final model ($p > 0.05$).

The response to a simulated predator by fish from the two incubation environments was analyzed by assigning each fish a binomial score of “reacted” or “did not react” depending on their response during the simulated attack. We used a generalized linear model with the reaction to the predator as a response variable, treatment as a fixed factor, and a logit link function for data with a binomial distribution. Another binomial

score was given of “found shelter” or “did not find shelter” and we used the same model as that described above but with the sheltering response as the response variable. If a fish found the shelter, the time elapsed from when it entered the shelter to when it reappeared was recorded. The count of seconds elapsed until re-emergence from the shelter of fish from the two incubation environments was analyzed with a generalized linear model with treatment (fixed) as the explanatory variable and a log link function for data with Poisson distribution.

At the onset of the semi-natural stream experiment, initial fork length and weight measurements were analyzed using a two-way ANOVA model with “stream” (random factor) and treatment (fixed factor) as explanatory variables. To analyze growth rates in semi-natural stream channels, both in terms of weight (*W*) and length (*L*), we used the following specific growth rate equation,

$$SGR = \frac{(ln\, Wfinal - \ln Winitial)}{Time}\ x\ 100$$

We did not individually mark fish and for this reason, we did not have the specific initial *W* or *L* for each individual surviving fish. Therefore, we calculated the average initial *W* or *L* of each treatment in a stream and used this value as the initial *W* or *L* in the equation. Thus, the *SGR* of an individual surviving from Stream A would be calculated from the average initial *W* or *L* of all fish from its respective treatment in Stream A. *SGR* was used as a response variable in a two-way ANOVA model with incubation treatment (fixed) and stream channel (random) as explanatory variables. The interaction treatment-by-stream was not significant (p = 0.45), and therefore removed from the model.

We used recapture information as a proxy for survival of fish released into semi-natural stream channels. If a fish was recaptured, it was assigned a value of 1. All other fish, i.e. those we did not capture, were assigned a value of 0. We analyzed the proportion of fish surviving (# survived / # recaptured) using a generalized linear model with binomial error and treatment as a fixed factor.

All statistical analyses were carried out using JMP Version 10.0 software. Underlying assumptions were tested based on residual plots and appropriate transformations (e.g. log transformation) were utilized if violations were detected.

Results

Body size and condition at emergence

We found that the addition of gravel to incubation units resulted in significant differences in body weight and condition but not body length (Table 1). Fry incubated in complex units were heavier than those incubated in simple units ($F_{1,208} = 11.02$, $p = 0.001$). Fry weight did not vary among replicates of the same treatment ($F_{1,5} = 1.69$, $p = 0.138$). Fork length was marginally non-significant between treatments ($F_{1,208} = 3.1$, $p = 0.080$), but did differ among replicates of the same treatment ($F_{1,5} = 3.48$, $p = 0.005$). As a result, fry emerging from complex incubation units had a significantly greater body condition value ($F_{1,208} = 39.7$, $p < 0.001$) than fish emerging from simple incubation units, i.e. fry from complex incubation units were heavier per unit length than fry emerging from simple incubation units. Condition did not vary among individual incubation units ($F_{1,5} = 1.44$, $p = 0.202$, Table 1).

Brain size analysis

We found that the addition of gravel to incubation units did not significantly affect neural development in terms of the volume of the total brain, telencephalon, and olfactory bulbs of Atlantic salmon fry. Log transformed absolute whole brain, telencephalon, and olfactory bulbs did not differ between treatment groups ($p > 0.6$; Table 2). Furthermore, no replicate effects were detected ($p > 0.1$). Additionally, no differences were detected in whole brain, telencephalon, and olfactory bulbs when controlling for either length (p > 0.60) or weight ($p > 0.1$; Table 2). Length was not a significant predictor of whole brain telencephalon volume ($p > 0.45$), but was a predictor of olfactory bulb volume ($p = 0.02$). By contrast, weight was a significant predictor of whole brain and telencephalon volumes ($p \leq 0.01$), but not of olfactory bulb volume ($p = 0.76$).

Behaviour experiments

Although individual fry from each incubation environment consumed novel prey during this experiment, there were significant differences in the total number of fry that fed between the two incubation groups. The number of fry that fed on prey from complex incubation environments was greater than the number of fry that fed on prey from simple incubation environments (df = 1, $p = 0.017$, Fig. 7). No arena effect was detected (df = 14, $p = 0.92$).

The behaviour of juvenile salmon to the simulated predator was variable. There was no difference in the number of fish that reacted when the predator was introduced between the two incubation environments ($\chi^2 = 0.50$, df = 1, $p = 0.48$). Of the 27 fish that reacted from complex incubation units (90.0%), 16 found the shelter and 11 did not and of the 32 fish that reacted from simple incubation units (84.2%), 20 found the shelter and

12 did not. There was no difference detected in shelter finding or number of fish that reappeared after finding shelter amongst fish from the two incubation environments (χ^2 = 0.06, df = 1, p = 0.79; χ^2 = 0.61, df = 1, p = 0.43, respectively). Of the fish that found the shelter, fish from the complex incubation environment took, on average, longer to emerge from the shelter than fish from the simple incubation environment (χ^2 = 68.3, df = 1, p < 0.01).

Growth and survival in a semi-natural environment

At the end of the experiment, we recaptured a greater number of fish originating from the complex than the simple incubation environments (χ^2 = 4.63, df = 1, p = 0.03, Fig. 8), indicative of a higher survival. The initial size of fry from the gravel incubation units introduced into the semi-natural streams was heavier and longer than that of the fry introduced from the simple incubation units (p < 0.05, Table 3). This size difference persisted over the 42-day experimental period, with the surviving fry from the gravel incubation units remaining heavier (p = 0.04) and longer (p = 0.03) than those from the simple units (Table 3). Moreover, the fry originating from complex incubation units grew significantly faster in terms of length than those from the simple unit over the 42 days ($F_{1,273}$ = 4.05, p = 0.04; Fig. 9), increasing the disparity in size. The fry from the two incubation environments did not differ in growth in terms of weight at the end of the 42 day experimental period, ($F_{1,273}$ = 2.22, p = 0.13).

Discussion

We found significant incubation environment effects on weight, behaviour, and subsequent performance in a semi-natural environment of emergent Atlantic salmon at

the start of exogenous feeding. These differences are likely to be environmentally induced, since fish from the two incubation treatments originated as a random pool of larvae from 85 crosses of adult Atlantic salmon. We found no differences between fish from the two incubation environments in fork length (marginally non-significant), whole brain or measured sub-structure (TE and OB) volume (in absolute terms and when corrected for body size). Fish reared in a complex incubation environment were heavier and in better condition at emergence when compared to fish incubated in a simple incubation environment. They also fed more readily on live prey and took longer to reappear from shelter after a simulated predator attack when compared to fish from the simple incubation environment. In semi-natural stream channels, more fish originating from complex incubation units survived the 42-d period than fish from simple incubation units. Moreover, of the fish that survived, complex fish experienced higher specific growth rates in terms of length compared to fish from simple incubation units.

Our results are consistent with previous investigations of the influence of incubation environment on emergent Atlantic salmon morphology, where fish incubated in gravel-filled incubation devices were heavier and in better condition than fish incubated without gravel (Leon and Bonney 1979, Hansen et al. 1985, Bamberger 2009a, b). However, whereas Bamberger (2009a, b) and Näslund et al. (2012) found enhanced fork length measurements in fish incubated in a gravel environment, we found a marginally non-significant difference (p =0.08). One explanation for these observed size differences could be more efficient allocation of endogenous energy reserves for growth by alevins (yolk sac larvae) incubated in a complex environment (Leon 1975, Hansen et al. 1985). Complexity in the incubation environment provides interstitial spaces where

salmon can rest. Indeed, Millidine et al. (2006) found that the presence of shelter significantly reduces maintenance metabolism of juvenile salmon. Fish incubated without any form of structure (such as those in the simple treatment in this experiment) tend to be more mobile than fish incubated in a structurally complex environment (Kihslinger and Nevitt 2006) possibly due to elevated numbers of interactions with conspecifics and constant effort expended at maintaining an upright position (Bams 1969). This could lead to less energy allocated to somatic growth and more allocated towards mobility and "righting" (Bams 1969, Benhaim et al. 2009). The body size difference observed in this experiment could be ecologically relevant since larger body size at this stage of the salmon life cycle could be advantageous in terms of territory establishment (Keeley and Grant 1995) and survival (Einum and Fleming 2000). Therefore, stocking fish out of simple incubation environments without gravel could put these fish at a disadvantage in the wild.

At emergence, juvenile salmon exhibit behaviours associated with entering the stream environment including transitioning to exogenous feeding and avoiding predators. Emergence is typically defined as a "critical period" marked with high levels of mortality predominately due to starvation and predation (Fleming and Einum 2011) and is a common life stage for captive-rearing releases (Maynard et al. 1995, Jonsson and Jonsson 2009). It is surprising, therefore, that experiments investigating behavioural traits of salmon at the emergence stage (i.e. originating from different incubation environments) is generally lacking. To our knowledge, this is one of the first studies to document the effect of the incubation environment on subsequent behaviour, in this case feeding and predatory response behaviour, following emergence in salmonid fish.

Fish incubated in a complex environment took longer to reappear from shelter following a simulated predator attack when compared to fish incubated in a simple environment. These observed results could suggest that fish incubated in complex environments are less prone to take "risks" in the face of a predator attack. If there is a high level of predation "risk" within the environment, which can be the case for emergent salmon fry, such behaviour could subsequently lead to enhanced survival. Roberts et al. (2011) observed similar results of juvenile Atlantic salmon at a later stage of early life, as 1+ Atlantic salmon reared in enriched tanks expressed less risk taking behaviour than those reared in conventional hatchery tanks. The results presented here suggest that changes within captive rearing environments can produce changes in behavioural phenotype of emergent Atlantic salmon which would potentially result in a survival advantage if released into the wild.

Fish incubated in a complex environment fed more readily on novel live prey when compared to fish incubated in a simple environment. In the wild, juvenile Atlantic salmon typically hold positions on or just above the gravel streambed facing upstream and dart up into the water column to capture drifting prey. Thus, position in the stream channel is a key component of prey capture. It has been observed that fish originating from experimental incubation environments with gravel hold more positive, upstream-facing feeding positions in flowing water (Bamberger 2009), and tend to move less than fish incubated without gravel (Kihslinger and Nevitt 2006, Benhaim et al. 2009). Such positioning may thus explain why the fish originating from complex incubation environments fed more readily than those incubated without complexity in a 90-s observation period. The 90-s observation period was chosen to reflect a fish's ability to

feed quickly on drifting prey and in the face of potential competition as would be experienced in the wild. This would presumably confer a growth and possibly survival advantage.

Brown et al. (2003) found that 5-month old Atlantic salmon reared in structurally enriched tanks displayed enhanced foraging abilities. Rodewald et al. (2011) also found that structural enrichment in hatchery tanks led to an enhancement of feeding abilities in Atlantic salmon of similar age. Both studies attributed this difference mainly to environmental enrichment (adding structure to rearing tanks) influencing cognitive abilities via neural plasticity. Structural complexity provides more stimuli in the environment, which could provide opportunity for enhanced neural development, as seen in rodents and other species (Falkenberg et al. 1992, review by van Praag et al. 2000, Mohammed et al. 2002). Because neurogenesis occurs throughout a fish's lifespan (Zupanc et al. 2005), it follows logically that adding enrichment to hatchery rearing environments could promote enhancement in neural development.

We investigated the effects of incubation environment on neural development in terms of brain volume. Multiple studies have documented plastic responses of fish brain size, brain cell proliferation, and gene expression to environmental enrichment (Marchetti and Nevitt 2003, Kihslinger and Nevitt 2006, Burns et al. 2009, von Krogh et al. 2010, Näslund et al. 2012, Salvanes et al. 2013). Our study is not consistent with some results observed in recent studies on the effect of incubation environment on brain size (see Kihslinger et al. 2006, Kihslinger and Nevitt 2006, Näslund et al. 2012). Using digital photographs and correcting for body length, Näslund et al. (2012) found that emergent Atlantic salmon incubated with structure had significantly larger whole brain and sub-

regions (olfactory bulbs, telencephalon, optic tectum, and cerebellum) than those incubated without structure. In the present study, we found no difference in either absolute or size-corrected whole brain and measured sub-structure volume based on histological sectioning of fish incubated in the two environments. Kihslinger and Nevitt (2006), using a similar measurement methodology to ours, found no differences in whole brain volume but relatively larger cerebellar volumes in steelhead trout (*Oncorhynchus mykiss*) incubated in trays with gravel versus fish incubated in trays without gravel. Unfortunately, we could not accurately differentiate the cerebellar region from the rest of the brain, which may be a function of sagittal versus transverse sectioning. Interestingly, Kotrschal et al. (2012) recently found hatchery reared juvenile coho salmon (*Oncorhynchus kisutch)* had larger brains compared to wild fish. These results, along with our own findings, call into question whether whole, or sub region brain volumes are the most appropriate measure of neural development when considering behavioural traits and performance of juvenile Atlantic salmon in semi-natural streams. Overall brain size does not necessarily reflect differences in neurological traits such as brain cell proliferation, number of neurons, or dendrite lengths (Mohammed et al. 2002, review by Healy and Rowe 2007), which may affect the performance of juvenile salmon. Environmental enrichment, in the form of complex structure, was found to significantly enhance cell proliferation in the telencephalon of zebrafish (von Krogh et al. 2010). Additionally, Lema et al. (2005) observed differences in cell proliferation in the telencephalon of coho salmon (*Oncorhynchus kisutch*) in response to environmental conditions and this difference did not generate differences in the size of the telencephalon. Most recently, Salvanes et al. (2013) found that exposing juvenile Atlantic

salmon to enriched tanks resulted in upregulation of genes in the forebrain and enhanced learning. Thus, it is clear that more studies are needed to understand this complex relationship between environment and neural development of juvenile salmon (reviewed by Ebbesson and Braithwaite 2012).

In semi-natural stream channels, we observed enhanced growth rates in terms of length and greater survival of fish originating from complex incubation environments. To our knowledge, these are novel findings since previous studies on incubation environment effects on juvenile Atlantic salmon phenotype lacked performance evaluations of the fish immediately after emergence (Bamberger 2009a, Bamberger 2009b, Näslund et al. 2012). Phenotypic differences of fish from differing incubation environments observed in this study (e.g. larger size, enhanced feeding, and response to a simulated predator) could explain differences in growth and survival in the semi-natural streams. These results suggest that, from an applied perspective, stocking of newly emergent fish that originated from simple incubation environments may put them at a disadvantage in the wild.

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Table 1. Mean + S.D. and ranges of body mass (*M*), fork length (*L*) and body condition (residuals from log *W* versus log *L*) of newly emerged *Salmo salar* fry incubated in simple and complex incubation units over the winter of 2011. *F* and *P* values are displayed for 2-way nested ANOVA tests across replicates of the same treatment and across treatments.

Treatment	*n*	*M* (g) Mean + S.D	Range	ANOVA (replicates, d.f. = 5)	ANOVA (treatments, d.f. = 1)
Simple	120	0.189 + 0.019	0.131 - 0.229	$F = 1.69$; $P = 0.14$	$F = 11.02$; $P < 0.01$*
Complex	90	0.199 + 0.019	0.140 - 0.232		

Treatment	*n*	*L (cm)* Mean + S.D	Range	ANOVA (replicates, d.f. = 5)	ANOVA (treatments, d.f. = 1)
Simple	120	2.96 + 0.08	2.7 - 3.1	$F = 3.48$; $P < 0.01$*	$F = 3.1$; $P = 0.08$
Complex	90	2.94 + 0.09	2.7 - 3.1		

Treatment	*n*	*Body Condition* Mean + S.D	Range	ANOVA (replicates, d.f. = 5)	ANOVA (treatments, d.f. = 1)
Simple	120	-0.012 + 0.04	-0.11 - 0.09	$F = 1.44$: $P = 0.21$	$F = 39.7$ $P < 0.01$*
Complex	90	0.016 + 0.03	-0.06 - 0.07		

Note: All measurements were log transformed before analysis. Asterisks (*) indicate significant results a the $p < 0.05$ level.

Table 2. Summary of ANOVA and ANCOVA results for emergent Atlantic salmon brain size incubated in two environments (n = 12 complex, n = 16 simple).

	Absolute ANOVA			
	Treatment (d.f. = 1)		Replicate (d.f. = 5)	
	F	*p*	*F*	*p*
Whole brain	0.18	0.673	0.54	0.742
Telencephalon	0.13	0.724	1.92	0.134
Olfactory bulbs	0.05	0.832	1.74	0.169
	Length ANCOVA			
	Treatment (d.f. = 1)		Length (d.f. = 1)	
	F	*p*	*F*	*p*
Whole brain	0.18	0.679	0.53	0.473
Telencephalon	0.12	0.729	0.54	0.470
Olfactory bulbs	0.09	0.762	6.61	0.016*
	Weight ANCOVA			
	Treatment (d.f. = 1)		Weight (d.f. = 1)	
	F	*p*	*F*	*p*
Whole brain	2.71	0.112	14.57	0.001*
Telencephalon	0.18	0.676	6.95	0.014*
Olfactory bulbs	0.09	0.759	3.02	0.094

Note: All measurements were log transformed before analysis. Asterisks (*) indicate significant results a the $p < 0.05$ level.

Table 3. Initial and final mean ± S.D. and ranges of body mass (*M*) and fork length (*L*) of newly emerged *Salmo salar* fry incubated in simple and complex incubation used for semi-natural stream channel experiment. *F* and *P* values are displayed for 2-way ANOVA tests across streams and across treatments.

		Initial *M* (g)			
Treatment	*n*	Mean ± S.D	Range	ANOVA (stream, d.f. = 7)	ANOVA (treatments, d.f. = 1)
Simple	320	0.187 ± 0.02	0.125 - 0.258	*F* = 2.94; *P* < 0 .01*	*F* = 25.99; *P* < 0.01*
Complex	320	0.195 ± 0.02	0.12 - 0.239		
		Initial *L (cm)*			
Treatment	*n*	Mean ± S.D	Range	ANOVA (stream, d.f. = 7)	ANOVA (treatments, d.f. = 1)
Simple	320	2.91 ± 0.08	2.7 - 3.1	*F* = 0.58; *P* = 0.77	*F* = 4.99; *P* < 0.05*
Complex	320	2.92 ± 0.08	2.6 - 3.1		
		Final M (g)			
Treatment	*n*	Mean ± S.D	Range	ANOVA (stream, d.f. = 7)	ANOVA (treatments, d.f. = 1)
Simple	122	0.39 ± 0.19	0.15 - 1.19	*F* = 9.27: *P* < 0.01*	*F* = 5.03; *P* = 0.026*
Complex	153	0.46 ± 0.27	0.12 - 1.66		
		Final L (cm)			
Treatment	*n*	Mean ± S.D	Range	ANOVA (stream, d.f. = 7)	ANOVA (treatments, d.f. = 1)
Simple	122	3.46 ± 0.44	2.8 - 5.0	*F* = 8.35: *P* < 0.01*	*F* = 4.23; *P* = 0.041*
Complex	153	3.59 ± 0.53	2.6 - 5.5		

Note: All measurements were log transformed before analysis. Asterisks (*) indicate significant results a the *p* < 0.05 level.

Figure Legends

Figure 1. Diagram of the incubation set-up used to rear Atlantic salmon eggs from the eyed egg stage through emergence. Arrows indicate direction of water flow. There was a temperature control failure in the upper-right "complex" replicate and it was removed from the experiment.

Figure 2. Cross-section of upwelling complex incubation unit. Arrows indicated direction of water flow.

Figure 3. Sagittal section of a juvenile Atlantic salmon brain. The olfactory bulb (OB) and telencephalon (TE) are labeled.

Figure 4. Diagram of behavioural observation arena (av. 18 x 11.5 x 5cm). Continuous water flow from the Saint John River entered and exited the arena through mesh screens at the upstream and downstream ends. For feeding trails, *Artemia* was injected into the arena through the middle of the upstream mesh screen. Dashed line represents water level.

Figure 5. Diagram of semi-natural stream channels (av. 4.6 x 0.45 x 0.27m). Continuous water flow from the Saint John River entered at the upstream end of the channel into a reservoir. Water spilled out of the reservoir and moved downstream through the stream channel and out through a screened barrier at the downstream end.

Figure 6. Juvenile Atlantic salmon (*Salmo salar*) marked with a visually implanted elastomer (VIE) tag at the dorsal and adipose fin. Only dorsal VIE tags were used for the semi-natural stream channel experiment to mark fish from each incubation treatment.

Figure 7. Percentage of emergent Atlantic salmon fry incubated in two environments observed to feed during 90-second trials following the presentation of novel prey. The number of fish tested was n = 38 simple fry and n = 38 complex fry. Asterisks (*) indicate significant results at the $p < 0.05$ level.

Figure 8. Percentage of fish that survived during the 42 day semi-natural stream channel experiment originating from complex and simple incubation environments. Asterisks (*) indicate significant results at the $p < 0.05$ level.

Figure 9. Specific growth rates (% / day) in terms of weight (top) and length (bottom) of Atlantic salmon fry in semi-natural stream channels from two incubation environments. Asterisks (*) indicate significant results at the $p < 0.05$ level.

Figure 1

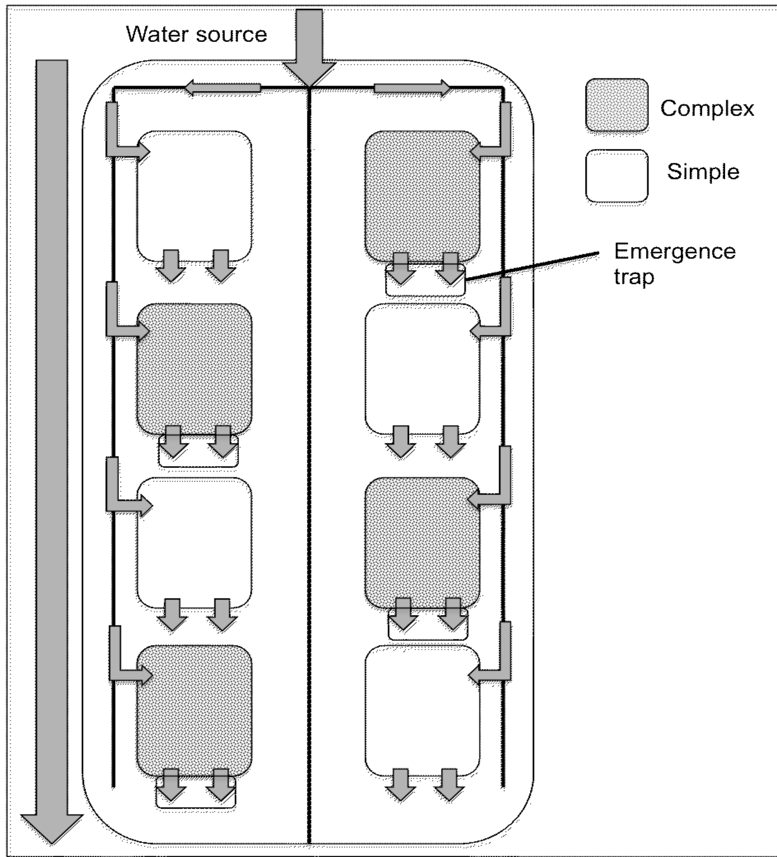


Figure 2

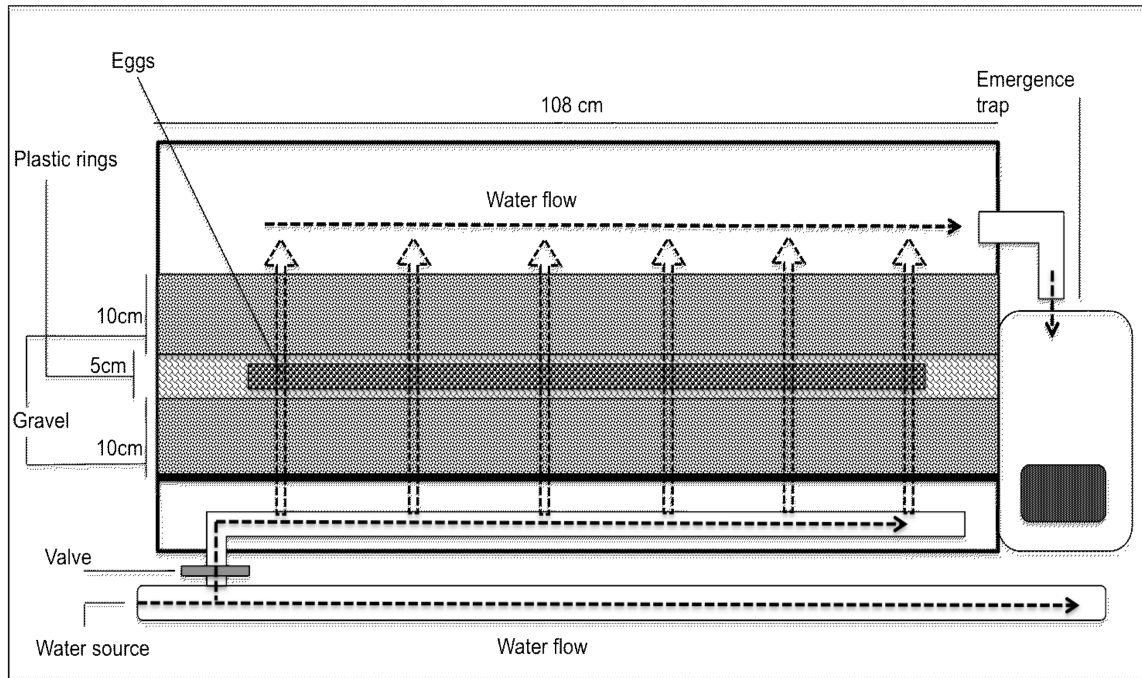


Figure 3

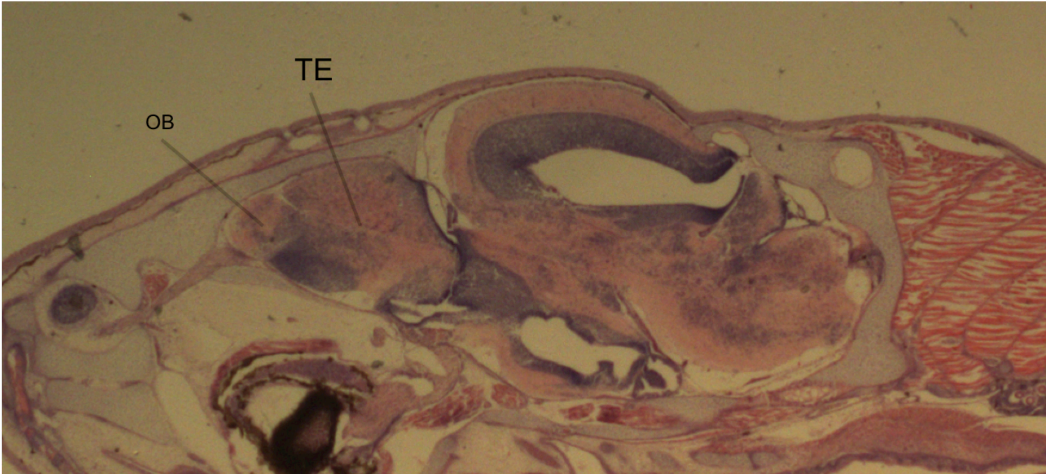


Figure 4

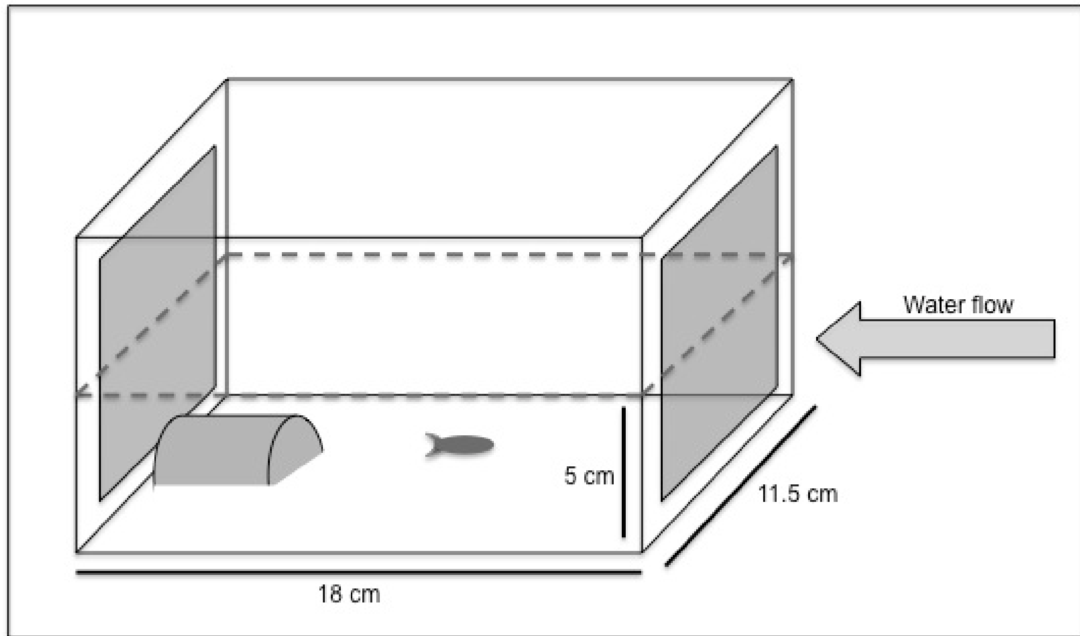


Figure 5

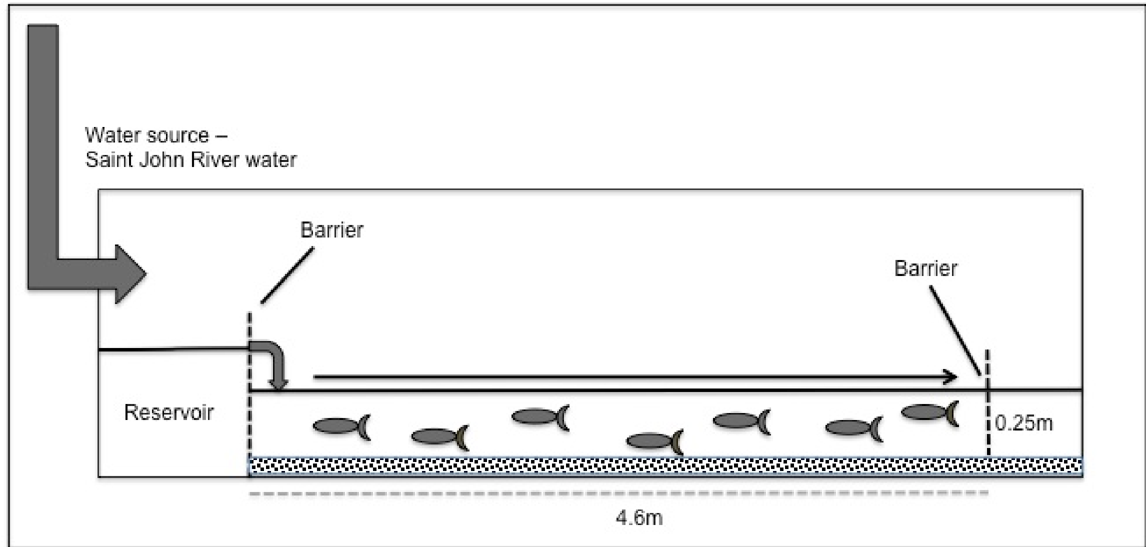


Figure 6

Figure 7

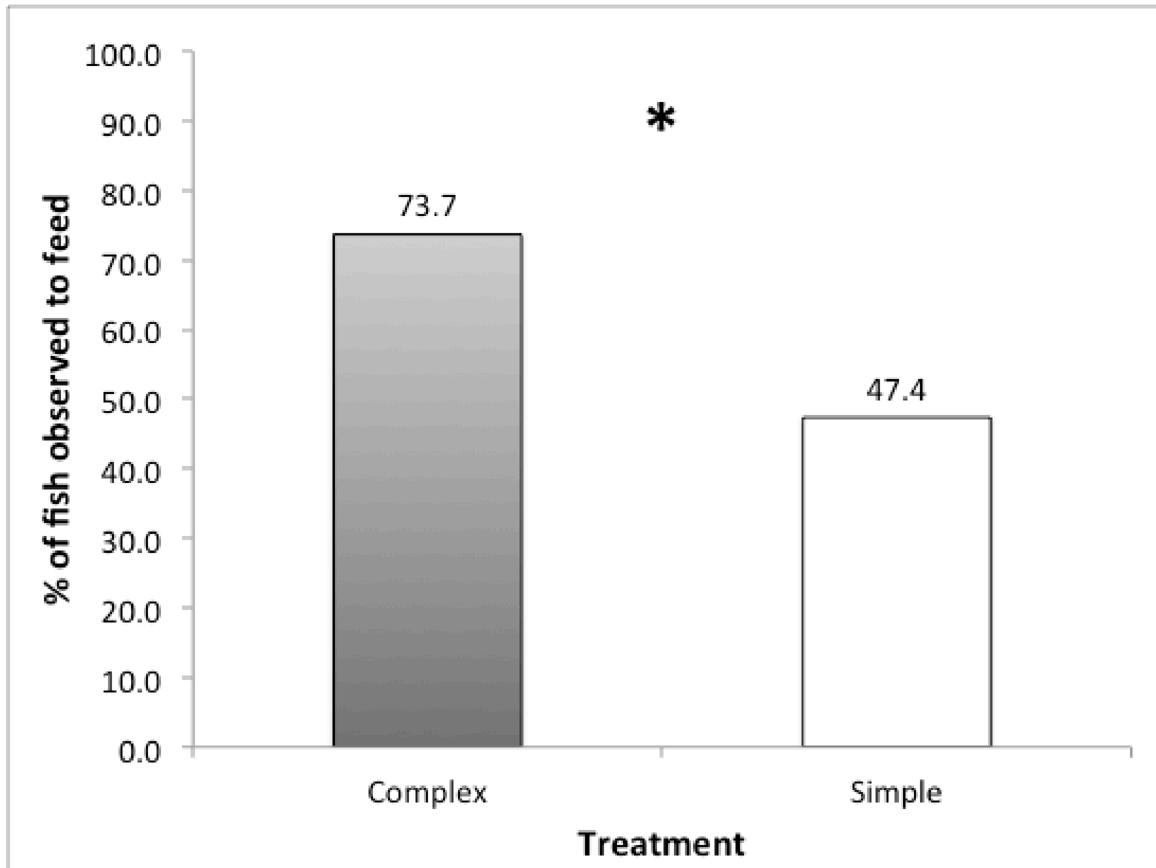


Figure 8

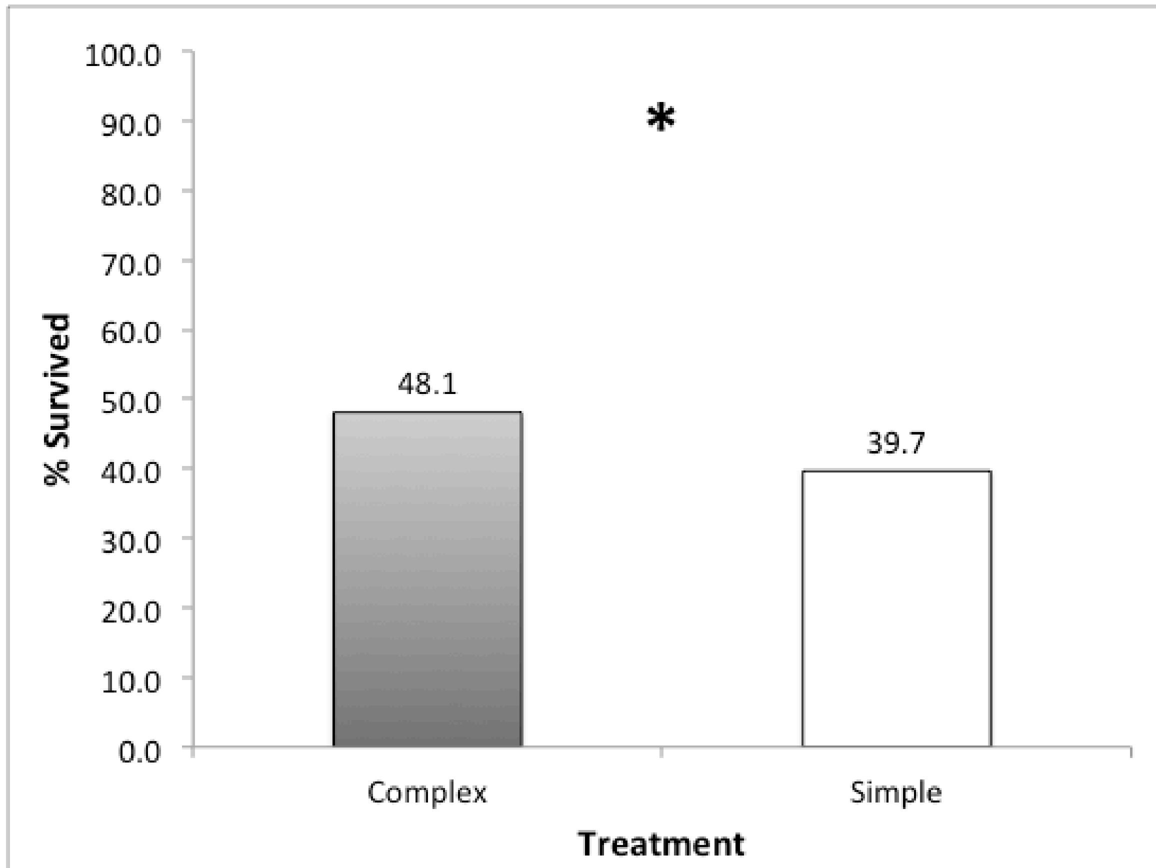
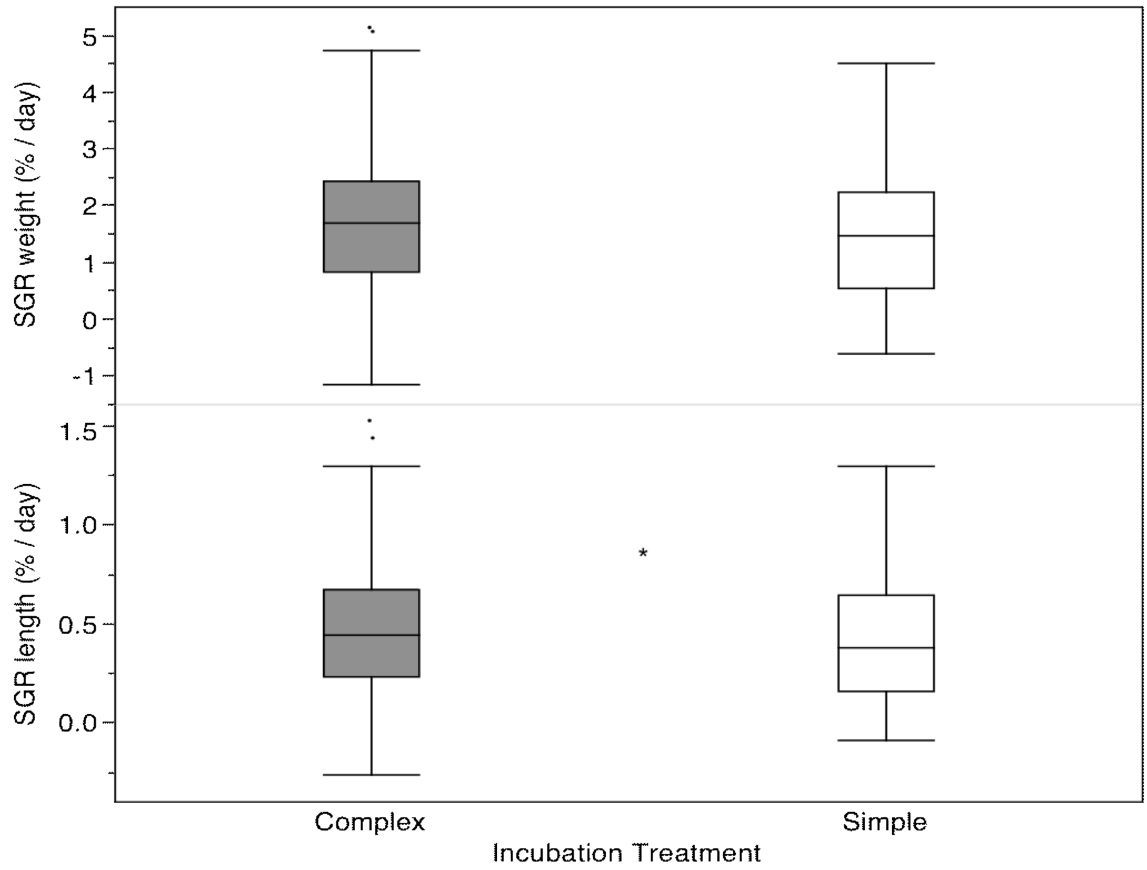


Figure 9



Chapter 3. Does early life exposure to structural complexity influence phenotype and performance of juvenile Atlantic salmon (*Salmo salar*)?

Abstract

When captive-reared animals are released into the wild they often perform poorly in terms of growth and survival. This deficiency could be mitigated by alterations in the captive rearing environments to better 'prepare' animals for life in the wild. In this study, we investigated the effects of exposure to an enriched environment (gravel and cobble added to rearing tanks) for different durations of time and at different points in early ontogeny on growth and condition of juvenile Atlantic salmon (*Salmo salar*). Then at 60 days post-emergence, fish from each treatment group were released into a semi-natural and a wild stream to evaluate performance. We used semi-natural stream channels to compare results with those found in our wild release experiment. At 30 d post-emergence, fish from the CC treatment (complex incubation and complex rearing for 30 d) – were heavier than all other fish. At 60 d post-emergence, fish from the CCS treatment (complex incubation, complex rearing for first 30 d and simple rearing for next 30 d) were larger than those from the CSS and SSC treatments. In semi-natural stream channels we sampled fish after over wintering (250 d after release) and did not detect a difference in survival amongst treatment groups. In the wild, we sampled 30 and 250 d (over winter) after release. At the first sampling event (30 d after release) we recaptured the highest number of fish from the SCS and CSS rearing treatments. At post-winter sampling we recaptured the highest number of fish from the CSS and SCC treatments. Results from release experiments also suggest a possible compensatory growth response of treatment groups with smaller fish before release, as they expressed higher growth rates in the semi-natural streams and in the wild. At the final recapture event (post-winter), these groups did not differ in terms of length, weight, or condition in either environment. Our results

suggest that habitat enrichment early in ontogeny can have a significant influence on morphology and post-release survival, but in some cases effects may not be all that straight forward.

Introduction

Fish are commonly reared in captivity and released into the wild for restocking purposes, including fisheries supplementation and biodiversity conservation. Often referred to as 'barren,' captive-rearing environments (e.g., hatcheries) are generally simple and lack the environmental influences that a fish would experience in nature. Thus, selective forces are strikingly different between these two environments and for this reason captive-reared fish can express remarkable phenotypic divergence from their wild counterparts (Einum and Fleming 2001, Huntingford 2004, Fraser 2008). It is not surprising, therefore, that captive-reared fish often perform poorly when released into wild environments (Maynard et al. 2004, Le Vay et al. 2007, Jonsson and Jonsson 2009).

To enhance performance of captive-reared fish in the wild, there is a growing trend of studies investigating alterations in fish rearing facilities to promote the expression of phenotypes that could be more favourable in nature (Aprahamian et al. 2003, Brockmark et al. 2010a, b; reviewed by Watters et al. 2003, Maynard et al. 2004, Brown et al. 2013). Several studies have suggested that adding habitat complexity to captive environments early in ontogeny can impact neural development (Kihslinger et al. 2006a, b, reviewed by Ebbesson and Braithwaite 2012, Näslund et al. 2012, Salvanes et al. 2013) and promote behavioural flexibility and learning in salmon and other fish species (Brown and Laland 2001, Salvanes and Braithwaite 2006, Strand et al. 2010). For

example, juvenile Atlantic salmon (*Salmo salar*) have expressed enhanced foraging abilities (Brown et al. 2003, Rodewald et al. 2011,), reductions in risk-taking behaviour (Roberts et al. 2011), and increased shelter-seeking behaviour (Appendix B) as a result of adding structural complexity to captive rearing environments in early stages of development. Furthermore, juvenile steelhead trout (*Oncorhynchus mykiss*) reared in tanks with structural complexity during the first year of development expressed social dominance over subordinates reared in conventional hatchery tanks (Berejikian et al. 2001). These observations give momentum to the current movement of refining captive-rearing techniques to promote phenotypic expression of traits that could be more favourable in nature (Maynard et al. 2004, Le Vay et al. 2007, Jonsson and Jonsson 2009).

While it is important to understand behavioural traits of captive-reared fish to identify mechanisms that might explain poor performance in nature (Tsukamoto et al. 1999, Salvanes and Braithwaite 2006), it is critical to conduct experiments in nature to assess how different captive-rearing techniques affect post-release performance.

The purpose of this study was to examine the effects of structural complexity on post-release performance of juvenile Atlantic salmon (age-0). We manipulated conventional captive rearing environments by adding gravel and cobble to rearing tanks and raised young-of-the-year Atlantic salmon in structurally 'enriched' environments or control environments (void of structural structure). We started experimental rearing during incubation (when eggs had developed eyes) and raised fish in complex (simulated gravel nests) and simple environments. Our results from a previous study (Chapter 2) suggest incubation in a gravel environment can produce larger fish in better condition,

can enhance foraging on live / novel prey, and increase performance (i.e. growth and survival) in semi-natural stream channels at emergence (i.e. the start of exogenous feeding). In this study, we aimed to look beyond the incubation / emergence stage. Therefore, we reared fish for two 30-day experimental periods following emergence where we introduced them to simple or complex (added cobble) rearing environments. This experimental design allowed us to investigate the influence of both 1) duration of exposure to enrichment and 2) period of early ontogeny exposed to enrichment on growth in captivity and post-release performance. At the end of the captive-rearing period, roughly 270 fish in each rearing treatment were individually marked and released into a nearby stream in early September 2011 and recaptured on two occasions (October 2011 and May 2012) to assess performance in nature. The remaining fish were marked and released in September 2011 in two semi-natural stream channels, and recaptured in May 2012. Semi-natural environments were used as a proxy for wild environments (Fleming and Einum 1997, Berejikian et al. 2001), but had lower predation risk and higher recapture efficiency (they could be drained). In a broad sense, our study addresses the impact of structural complexity, i.e. a form of environmental enrichment, in fish rearing facilities on post-release performance of juvenile Atlantic salmon.

Methods

Experimental Fish

Gametes were collected from 85 female and 85 male Atlantic salmon (*Salmo salar*) from the Tobique River, NB (46.8°N, 67.8°W; Fig. 1) in November 2011.

Adults were captured in the Tobique River during their seaward migration as smolts and were reared for 1.5 years to sexual maturity at the Mactaquac Biodiversity Centre (MBC; Fig. 1), Department of Fisheries and Oceans, New Brunswick, Canada, as part of a salmon enhancement program. Once eggs were fertilized and became "eyed" (i.e. had developed visible eyes), 4,000 eggs were haphazardly assigned to each experimental incubation unit on 18 February 2011, where they reared until emergence (i.e. the start of exogenous feeding). We aimed to minimize genetic and other parental effects by haphazardly distributing the fertilized eggs from the common pool of crosses (Kennedy et al. 2008).

Captive Rearing

Experiments began during incubation at the eyed-egg stage (i.e. when fertilized eggs developed eyes). Eyed eggs were incubated in eight individual units (108 x 55.2 x 44.5 cm) consisting of 4 replicates of each of two environments (simple and complex) and were positioned in an alternating fashion over a conventional egg trough. The four 'complex' incubation units were filled with 10cm of gravel (ca. 3.75mm in diameter), 5 cm of semi-hollow plastic rings (5 x 2.5 cm) as an incubation medium where eggs were placed, and topped with an additional 10cm of gravel. The four 'simple' incubation units were identical except they contained no substrate of any kind (See Chapter 2 for full description of the incubation environments).

Rearing post-incubation (Stage 1 & 2)

Rearing post-incubation was divided into two 30-day rearing stages; 1) emergence to 30 d post-emergence and 2) 31 to 60 d post-emergence. Ambient Saint John River water

flowed into 1m circular tanks at a average of 0.2 L • s^{-1} and depths averaged 14.6 cm ± 0.37 (range 14.0 – 15.0 cm). Rearing tanks were categorized as either 'simple' (S) or 'complex' (C). Complex tanks had cobble (Mean = 13.5 cm; range 8 – 16 in diameter) covering roughly 80% of the bottom and simple tanks were void of structure (Fig. 2). Fish were fed *ad libitum* a mixture of pellets, dried krill, and fish oil throughout the rearing period (post-emergence).

From 15 – 17 June 2011 (emergence, i.e. 'swim-up'; see Chapter 2), groups of unfed fry (about to begin exogenous feeding) from each incubation unit were netted haphazardly from incubation units, counted in groups of 1000, and introduced to the 1m circular tanks for post-emergence rearing (Stage 1). Each group of 1000 fish originating from simple incubation units, having a numerically identical mix of individuals from the 4 replicates, was introduced to either a simple or complex rearing tank. The same method was employed for groups of fish originating from complex incubation units. Therefore, four treatment groups were created originating from the two incubation environments (simple incubation—simple Stage 1 rearing [SS], simple incubation—complex Stage 1 rearing [SC], complex incubation—complex Stage 1 rearing [CS], and complex incubation—complex Stage 1 rearing [CC]) and 4 replicate tanks were used for these new treatment groups (Fig. 3). Mortality was relatively high amongst treatments after introduction to rearing tanks (transitioning to first feeding) but subsided after 7-10 d. For this reason, SC and CC treatments had to be reduced to 3 replicates to maintain equal densities across all replicates. Subsequently, ca. 400 fish were reared in each tank for Stage 1.

At the end of Stage 1 (19 July 2011), fish from each rearing tank were counted and 145 were introduced to each of a simple and a complex rearing tank where they reared for the next 30 d (until 19 August 2011). Therefore, eight rearing treatments (3-4 replicates of each) were created at Stage 2 that derived from the four Stage 1 treatments originating from two incubation environments (simple-simple-simple [SSS], SSC, SCS, SCC, CSS, CSC, CCS, CCC; Fig 3). Mortalities were removed and recorded 2-3 times per week (simultaneous with tank cleaning) and fish densities were equalized across tanks over the course of the rearing period by netting and removing individuals haphazardly from tanks with higher densities.

Sampling – Growth in the hatchery

At the end of each sampling stage, 30 fish from each replicate were netted haphazardly, anesthetized with tricaine methanesulfonate (MS222), measured for fork length (L_F) and weight (W) and the left side of each fish photographed with a NIKON camera (D5100 with Micro Nikko 60 mm lens). Fish were not fed the day of sampling. All fish handling was performed under the protocol of the Canadian Council on Animal Care and was approved by Memorial University of Newfoundland's Institutional Animal Care Committee (protocols 11-18-IF and 12-18-IF).

Tagging and Release of Fish

From 23 August – 5 September 2011, 167 – 175 fish (> 4.5 cm) from each treatment (i.e. 40 – 50 fish per replicate tank) were lightly anesthetized (MS222), measured for fork length (cm) and weight (g) and inserted with a 8 mm passive integrated transponder (PIT) tag. Tags were inserted on the left side of each fish in the body cavity through a small

incision made by a scalpel in the body wall. Fish that were < 4.5 cm were marked with an visual implanted elastomere tag (VIE) indicating treatment group but were too small to PIT tag (n = 95 – 113 from each treatment group). These fish were used in survival analyses but not growth analyses. After tagging, fish were held at the hatchery for at least 72h to monitor for any effects that the tagging process may have had on fish health. All fish were in good condition during this observation period and did not show signs of irregular behaviour. Fish were then transported in insulated, oxygenated tanks to Quaker Brook, which was 160 km from MBC (Fig. 1), and released on 12 September 2011. In total, 2193 individual fish were released. Oxygen levels and temperatures were monitored periodically throughout transport. The release stream was chosen based on salmon habitat, accessibility, origin of fish used in this study, and approval by the Department of Fisheries and Oceans (DFO), Canada. Quaker Brook is a tributary of the Tobique River, New Brunswick (46.77°N, 67.6°W). Based on an initial electrofishing survey, presence of Atlantic salmon (< 175mm) and brook trout (*Salvelinus fontinalis*) were detected (amongst other, non-predatory fish species). Additionally, common mergansers (*Mergus merganser*), great blue heron (*Ardea Herodias*), and belted kingfishers (*Megaceryle alcyon*), all known predators of salmonids, were observed. Two release sites were chosen, roughly 150 m apart, and fish were divided into two release groups having a similar mix of individuals from treatments and replicates.

We sampled Quaker Brook on two occasions (11-12 October 2011 and 26 – 28 May 2012; roughly 28 and 260 d after release, respectively) via electrofishing. We electrofished from the mouth of the creek to 750m above the most upstream fish capture (roughly 1.8 km of stream). All recaptured fish were lightly anesthetized, scanned for PIT

tags, and measured for fork length (cm) and weight (g). Fish were re-released into Quaker Brook after the first sampling period (October 2011) and sacrificed with an overdose of MS222 if captured during the second sampling period (May 2012) for future neural analysis.

In addition to the wild release experiment, we released fish into semi-natural stream channels at MBC. We aimed to compare the results of a semi-natural stream experiment, where recapture efficiency approaches 100%, with those of the wild. A total of 450 fish were marked with PIT tags and 276 were marked only with VIE tags, measured for fork length and weight and released into two semi-natural stream channels at the hatchery. Each stream dimension averaged 31 m length and 3.1 m in width (Figure 4). The streambed was covered with gravel and cobble substrate, water velocities ranged from 1 to 72 cm • s^{-1}, and depths ranged from 12 to 60 cm (depth average = 35 cm). Therefore, an average of 87 m^2 of habitat was present in the stream channels. Each stream was consistent in its make up of riffles and pools. Species of aquatic invertebrates were abundant in each stream, providing food for juveniles. Only natural prey items were available to fish after release.

Sampling (via electrofishing) of the semi-natural stream channels occurred on 25 May 2011, roughly 260 d after release. Streams were electrofished, drained, and inspected visually for surviving fish. All recaptured fish were lightly anesthetized, scanned for PIT and elastomere tags, and measured for fork length (cm) and weight (g).

Statistical analysis

Treatment effects on body size (log weight [W] or log fork length [L_F]) at emergence (reported in Chapter 2), the end of Stage 1, and the end of Stage 2 were analyzed using a two-way nested ANOVA model where replicate tank (random factor) was nested within treatment (fixed factor). To examine body condition at each sampling period, residuals were produced from a regression analysis of log L_F (x-axis) and log W (y-axis) measurements and used as a response variable in a two-way nested ANOVA model where replicate tank (random factor) was nested within Treatment (fixed factor). A Tukey's HSD post-hoc test was performed to compare multiple means of treatment groups at the end of Stage 1 and Stage 2.

For the wild release experiment, growth rates were calculated for individual PIT tagged fish recaptured in October 2011 and for individual PIT tagged fish recaptured in May 2012. For the semi-natural stream experiment, growth rates were calculated for individual PIT tagged fish captured in May 2012. Growth rates in both the semi-natural stream channels and the wild were analyzed using two one-way ANOVA models with treatment as a fixed factor. Because growth rate varies as a function of size in Atlantic salmon (Jonsson and Jonsson 2011), we standardized growth (hereafter referred to as mass standardized growth rate) of individual fish released in the wild and semi-natural stream using the following equation (Ostrovsky 1995):

$$\Omega = \frac{M_2^{\tau} - M_1^{\tau}}{\tau \ x \ time}$$

where M_2 and M_1 are final and initial weights, time is the growth interval (difference in days from day of release to recapture), and τ is the species-specific coefficient for the

relationship between growth rate and size. We chose $\tau = 0.31$, as it is an accepted value for Atlantic salmon (Elliot and Hurley 1997, Forseth et al. 2011, Jonsson and Jonsson 2011). A Tukey's HSD post-hoc test was performed to compare multiple growth rate means of treatment groups. Finally, we compared the final length, weight, and condition of treatment groups in the semi-natural stream channels and in the wild using two one-way ANOVA models with treatment as a fixed factor.

We used recapture information as a proxy for survival of fish released into the wild and semi-natural stream channels. If a fish was recaptured, it was assigned a value of 1. PIT tagged individuals that we did not capture in October 2011 but did capture in May 2012 were included in the survival analysis for October 2011 because they must have been alive (Carlson et al. 2004). We analyzed the proportion of fish surviving (# recaptured / # marked and released) using a generalized linear model with binomial error and treatment as a fixed factor. We used this model to compare survival between all treatment groups. We also used two-way ANOVA models to compare the initial weight, length, and condition of fish that survived to the initial size of those that did not. We used treatment and survival (yes or no) as fixed factors. The interaction term "treatment*survival (yes or no)" was removed from all of the final models because it was not significant ($p > 0.05$).

All statistical analysis were performed using JMP software version 10.0 and $p < 0.05$ was chosen as the level of significance. Underlying assumptions were tested based on residual plots and appropriate transformations (e.g. log transformation) were utilized if violations were detected.

Results

Effects of rearing treatment on size

We found that the addition of gravel to incubation units resulted in significant differences in body weight and condition but not fork length at emergence (see Chapter 2). Fry incubated in complex units were heavier and had a significantly greater body condition value, i.e. were heavier per unit length, than fry emerging from simple incubation units.

At the end of Stage 1 (30 d post-emergence), we detected significant differences in body size and condition amongst treatment groups, with fish from CC being heavier than all other treatments and those from CS being lighter (Table 1). Weights also differed significantly amongst replicates. In terms of fork length, fish from CC, SC and SS were not different from one and other, but were significantly longer than fish from CS (Table 1). Given the differences in weight and length, body condition was lowest amongst fish from SS, did not differ amongst fish from CC and SC nor SC and CS, but did differ between CC and CS (Table 1). Overall, fish from CC tended to be the largest and have the highest body condition, while those from CS and SS tended to be the smallest and have the lowest body condition, respectively.

At the end of Stage 2 (60 d post-emergence), we detected significant differences in body size but not body condition amongst rearing paths. Fish from CCS tended to be the largest amongst the rearing paths, being significantly heavier and longer than fish from CSS and SSC, which tended to be the smallest amongst the rearing paths (Table 2, Fig. 5). All other treatments were not significantly different in terms of weight or fork length. We did not detect differences in body condition amongst treatments, i.e. fish from

all treatments did not differ with respect to their weight to length ratio (Table 2). There were significant replicate effects throughout (Table 2).

Wild release

Of the 2,182 tagged fish (1366 PIT and 816 VIE) released into Quaker Brook, we recaptured 177 PIT and 118 VIE tagged fish in October 2011 and 78 new PIT tagged fish (i.e. fish not caught in October but must have been alive) in May 2012. Nine fish were recaptured during both electrofishing events (n = 2 SCS, 1 SCC, 1 CCS, 1 CCC, 3 CSS, 1 SSC). Therefore in total, a minimum of 15.8 % (n = 344) of released fish were alive in October 2011. In addition to the 87 PIT tagged fish recaptured in May 2012 (n = 78 new and n = 9 recaptured in October 2011 and May 2012), we caught 88 VIE tagged fish during this same efishing event. Therefore, we recaptured 8.0 % of fish (n = 175) in May 2013 (Table 3). For our first sampling effort, we detected a significant difference in the number of fish recaptured between treatment groups (Table 3, $\chi^2 = 17.2$, df = 7, $p = 0.02$). We recaptured significantly more fish from CSS when compared to CCC, CSC, SSS, and SSC (Table 4). In addition, we caught more fish from SCS and SCC when compared to CSC (Table 4). Upon post-winter sampling, in May 2012, we detected a significant difference in the number of fish recaptured between treatment groups (Table 3, $\chi^2 = 15.4$, df = 7, $p = 0.03$). We caught significantly more fish from CSS when compared to CCC, CSC, and SSC. In addition we caught more fish from SCC when compared to CCC, CSC, and SSC (Table 5). There was no difference in the initial weight, length, or condition of PIT tagged fish that were recaptured versus those that were not (all $p > 0.05$, Table 6).

When comparing mass standardized growth rates in the wild, significant differences were detected amongst treatments for both sampling periods (Table 3). During

our first sampling event (October 2011), fish from CSS, SSS, SCC, and CSC, had significantly greater specific growth rates compared to fish from SSC, CCC, CCS, and SCS. During our second sampling event (May 2012), fish from CSS, SCC, and SSS outgrew fish from SCS and CCS. Additionally, fish from CSC outgrew fish from CCS (Table 3). By the end of the second sampling period in May 2012, we did not detect any significant differences in final weight, length, or condition amongst treatments (all $p > 0.1$, Table 5).

Semi-natural stream release

Of the 726 tagged fish released into the two semi-natural stream channels at MBC in September 2011, we recaptured 621 (85.5%) in May 2012 (n = 317 and 304 for Stream 1 and 2 respectively). Unlike our wild release, we did not detect a difference in survival between treatment groups ($\chi^2 = 0.79$, df = 7, $p = 0.33$, Table 8) nor did we detect a difference between streams ($\chi^2 = 0.08$, df = 1, $p = 0.77$). In contrast to the wild release, PIT tagged fish that survived overwinter in semi-natural stream channels were significantly smaller at the time of release than fish that did not survive, both in terms of weight and length ($F_{1,448} = 5.2$, $p = 0.02$ and $F_{1,448} = 6.6$, $p = 0.01$, respectively) but not in body condition ($F_{1,448} = 0.2$, $p = 0.6$, Table 9).

When comparing mass standardized growth rates in the semi-natural stream channels from September 2011 – May 2012, a similar pattern was evident as observed in the wild, i.e. fish from CSS, SSS, SCC, and CSC outgrew fish from SSC, CCC, CCS, and SCS (Table 8). Moreover, we did not detect a difference amongst treatment groups in final weight, length, or body condition (all $p > 0.7$, Table 10).

Discussion

To the best of our knowledge, the approach taken here is a novel design to test the influence of structural complexity on post-release performance of captive-reared Atlantic salmon. During the captive-rearing period, we detected differences in body size and condition in response to adding or removing structural complexity at different points in early ontogeny and for different durations of time. We also detected differences in growth rate and survival amongst treatment groups in the wild.

Our study revealed significant variation in body size and condition of juvenile Atlantic salmon in response to adding or removing structural complexity to rearing tanks at emergence and 30-days after emergence (Stage 1), however these differences generally faded by day 60 of captive rearing (Stage 2). Fish emerged from gravel incubation environments heavier and in better condition than fish incubated in simple incubation environments (see Chapter 2; see also Hansen et al. 1985, Bamberger 2009a, 2009b). After emergence, fish were reared in complex or simple tanks for 30 d. At the end of this stage of rearing, fish from complex incubation environments that were reared in complex tanks were significantly heavier than all other rearing groups. It has been suggested that fish reared in barren tanks tend to be more mobile than fish reared in structurally enriched tanks (Kihslinger and Nevitt 2006, Benhaim et al. 2009). Energy expended for extra swimming efforts in barren tanks could lead to less energy allocation for somatic growth and therefore smaller body size, as documented in our incubation study (Chapter 2) and in previous studies (Bams 1969, Hansen 1985, Bamberger 2009, Benhaim et al. 2009). Furthermore, fish reared in barren tanks have been observed to have elevated basal cortisol levels when compared to fish reared in structurally ‘enriched’ tanks (Näslund et

al. 2013). High cortisol levels, often associated with stress, have been blamed for negative effects on growth of salmon in captive environments (Pickering 1993, Basrur et al. 2010).

The size difference observed in the hatchery was much less pronounced amongst groups at the end of 60 days post-emergence (Stage 2) and there were no differences in body condition among treatments. Density may have played a significant role in this observation. The densities of fish in rearing tanks were reduced by half for Stage 2 due to splitting groups of fish after Stage 1. Since fish were larger at the end of Stage 2, we estimated the final biomass of fish in each tank and found that it was reduced by roughly 23% (Stage 1 = 1.32 g • L^{-1} and Stage 2 = 1.07 g • L^{-1}). Brockmark et al. (2010a, b) found fish density in rearing tanks to be more of an influence than adding structure on body size and condition in a captive setting. Thus, reduction of density and biomass could have reduced effects of crowding and stress and subsequently caused rearing conditions to become more favourable. Moreover, it could have allowed for a compensatory growth response, as differences in size and condition among treatment groups disappeared. Accelerated growth following a period of growth suppression due to unfavourable environmental conditions is not uncommon in juvenile salmon (Mortensen and Damsgard 1993, Maclean and Metcalfe 2001, Morris et al. 2011).

Environmental enrichment, in the form of adding structural complexity to rearing environments, has been observed to influence post-release performance in salmonids. Tipping (1998) observed enhanced return rates of sea-run cutthroat trout reared as juveniles in gravel bottom tanks. Additionally, enhanced return rates were observed for sea-run cutthroat trout reared for 4-7 months as opposed to only 1 month in gravel bottom tanks (Tipping 2001). In the present study, we detected differential survival amongst

treatment groups in the wild but not semi-natural stream channels. Approximately one month after release, we found that fish from CSS were recaptured at higher rates when compared to CCC, CSC, SSS, and SSC. During this same sampling period we recaptured more fish from SCS and SCC when compared to CSC. Upon post-overwinter sampling (i.e. 260 d after release), CSS fish maintained a higher recapture rate when compared to CCC, CSC, and SSC but not SSS. In addition, we caught more fish from SCC when compared to CCC, CSC, and SSC. There was no difference in survival detected between CSS and SCC for either recapture events. Body size has been suggested to be a critical factor influencing post-release survival of juvenile salmon (Quinn and Peterson 1996), however, CSS and SCC fish were significantly smaller than groups with lower survival (i.e. CCC) at the time of release. Furthermore, we did not detect a significant difference in the initial weight, length or body condition between fish that survived and did not survive, regardless of treatment group. Thus, larger fish did not survive more than smaller fish in this study. Overall, groups with differential survival overlapped in both the duration and timing of exposure to complex rearing environments and thus, it is difficult to draw a conclusion linking simple or complex rearing with survival. We did find that at emergence fish incubated in a gravel environment were larger and expressed superior growth and survival in semi-natural stream channels (See Chapter 2). Taken as a whole, however, gravel incubated fish did not do better on average (86 survivors) when compared to non-gravel incubated fish (89 survivors) in the wild in this experiment. These results suggest that incubation environment may have a significant influence on performance if released immediately after emergence but this influence may not carryover through subsequent rearing.

Adding structural complexity to hatchery rearing environments early in development has been observed to reduce maladaptive risk-taking behaviour (Roberts et al. 2011), increase shelter-seeking behaviour (Appendix B), and enhance foraging abilities of juvenile salmon (Brown et al. 2003, Rodewald et al. 2011). Subsequently, it has been observed that rearing salmonids in such a way during the first year of development can increase growth rates after release into semi-natural environments (Berejikian et al. 2000, 2001). We detected consistent patterns in growth rates amongst treatment groups in the wild and semi-natural stream channels, although differences varied in their level of significance. In this study, the treatment groups that expressed higher growth rates overlapped in their exposure to structural complexity, both in terms of timing and duration of timing, with those expressing lower growth rates. Interestingly, adding habitat complexity to rearing environments after emergence seemed to not influence post-release survival as fish that incubated in a complex environment but reared for the rest of the hatchery rearing period in a simple environment had higher recapture rates than fish with more exposure to habitat complexity. Therefore, the effect of habitat complexity on post-release growth and survival, both in terms of timing and duration of timing of exposure, remains ambiguous. We did, however, find that treatment groups with higher growth rates in both wild and semi-natural environments were smaller, in terms of body size, at the time of release. Upon recapture during the final sampling period, the surviving fish did not differ in length, weight, or condition among treatment groups in either environment. Therefore, the smaller fish likely expressed a compensatory growth response following release into the wild and semi-natural environments (Alvarez and Nicieza 2005, Johnsson and Bohlin 2005, Brockmark et al. 2007). It is possible that the

smaller size of fish at the time of release could reflect poor performance in a captive setting. In such a setting, fish are fed to satiation typically and often in a predictable location. Thus, bold and aggressive fish often express superior performance in such conditions (Huntingford 2004). In wild environments, however, food is limited and not always accessible in the same location. Therefore, fish with traits favourable in a captive setting, i.e. those that grew larger in captivity, may perform poorly in the wild (as observed by Saikkonen et al. 2011).

It could be suggested that compensatory growth may be accompanied by increased costs or risks. Fish that need to feed more could occupy habitat that is more susceptible to predation more often than occupying habitat with cover. Although it was not always the case, we found that fish with higher growth rates also expressed higher survival. For example, for our first recapture event in the wild we caught more fish from CSS when compared to SSC, CCC, and CCS. CSS fish also had significantly greater growth rates. It is possible that we recaptured more fish from CSS because they were occupying habitat that made them more susceptible to capture or predation (i.e. feeding areas and not cover). If they were more susceptible to predation, it would be likely that we would have caught less fish from this group during our second recapture event. We did not observe less fish from CSS during our second sampling period.

In conclusion, we observed significant differences among treatment groups during captive rearing in body size and condition at emergence and 30 d post-emergence. Observed size and body condition differences generally faded 60 d after emergence likely due to a compensatory growth response as the density and biomass in rearing tanks was reduced creating more favourable conditions across treatments. Overwinter survival in the

wild of fish that incubated in a complex environment and reared in a simple environment for 60 days was significantly higher than all other rearing treatment groups. This finding was not consistent with our results from the semi-natural stream channels at the hatchery, as we did not detect a difference in survival amongst treatment groups. We also found that releasing fish into semi-natural stream channels directly out of complex incubation environments resulted in higher survival and growth rates (see Chapter 2). We observed a possible compensatory growth response of smaller fish in the wild and semi-natural streams as there were no differences in final size or condition of recaptured fish. Our results suggest that it is possible to influence juvenile salmon development and post-release survival by manipulating captive rearing environments at the incubation stage.

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Table 1. Mean ± S.D. and ranges of body mass (*M*), fork length (*L*) and body condition (residuals from log *W* versus log *L*) of *Salmo salar* fry reared under four different experimental treatments at 30 d post-emergence. *Treatment* column includes incubation environment — rearing environment first 30 d post-emergence (e.g., complex [incubation] – complex [first 30 d post emergence]). *F* and *P* values are displayed for 2-way nested ANOVAs across replicates of the same treatment and across treatments. Tukey's HSD post-hoc test was performed to compare multiple growth rate means of treatment groups. Levels not connected by the same letter are significantly different.

		Weight (g)				
Treatment	*n*	Mean ± S.D	Range	Significant differences	ANOVA (replicates, d.f. = 10)	ANOVA (treatments, d.f. = 3)
Complex - Complex	90	0.39 ± 0.11	0.16 - 0.67	A	$F = 2.31$; $P = 0.01$*	$F = 14.89$; $P < 0.01$*
Simple - Complex	90	0.34 ± 0.11	0.15 - 0.70	B		
Simple - Simple	120	0.32 ± 0.09	0.15 - 0.61	BC		
Complex - Simple	120	0.30 ± 0.09	0.15 - 0.57	C		

		Fork length (cm)				
Treatment	*n*	Mean ± S.D	Range	Significant differences	ANOVA (replicates, d.f. = 10)	ANOVA (treatments, d.f. = 3)
Complex - Complex	90	3.4 ± 0.3	2.9 - 3.9	A	$F = 1.76$; $P = 0.07$	$F = 9.8$; $P < 0.01$*
Simple - Complex	90	3.3 ± 0.3	2.8 - 4.1	A		
Simple - Simple	120	3.3 ± 0.3	2.9 - 4.0	A		
Complex - Simple	120	3.2 ± 0.3	2.8 - 4.0	B		

		Body Condition				
Treatment	*n*	Mean ± S.D	Range	Significant differences	ANOVA (replicates, d.f. = 10)	ANOVA (treatments, d.f. = 3)
Complex - Complex	90	0.020 ± 0.033	-0.101 - 0.080	A	$F = 1.7$; $P = 0.08$	$F = 16.6$; $P < 0.01$*
Simple - Complex	90	0.012 ± 0.041	-0.083 - 0.104	AB		
Simple - Simple	120	-0.020 ± 0.040	-0.169 - 0.060	C		
Complex - Simple	120	-0.004 ± 0.056	-0.308 - 0.244	B		

Table 2. Mean ± S.D. and ranges of body mass (*M*), fork length (*L*) and body condition (residuals from log *W* versus log *L*) of *Salmo salar* fry reared under eight different experimental treatments at 60 d post-emergence (two environments by three stages). *Treatment* column includes incubation environment — rearing environment first 30 d post-emergence — rearing environment 31-60 d post-emergence (i.e. complex [incubation] – complex [first 30 d post emergence] – complex [second 30 d post-emergence]). *F* and *P* values are displayed for 2-way nested ANOVAs across replicates of the same treatment and across treatments. Tukey's HSD post-hoc test was performed to compare multiple growth rate means of treatment groups. Levels not connected by the same letter are significantly different.

		Weight (g)				
Treatment	*n*	Mean ± S.D	Range	Significant differences	ANOVA (replicates)	ANOVA (treatments)
CCC	90	0.92 ± 0.26	0.33 - 1.48	AB	$F_{20,805} = 1.97$; $P < 0.01$*	$F_{7,833} = 2.48$; $P = 0.02$*
CCS	90	0.93 ± 0.20	0.57 - 1.39	A		
CSC	120	0.86 ± 0.26	0.34 - 1.53	AB		
CSS	120	0.84 ± 0.26	0.41 - 1.65	B		
SCC	90	0.88 ± 0.29	0.31 - 1.91	AB		
SCS	90	0.89 ± 0.24	0.43 - 1.62	AB		
SSC	120	0.85 ± 0.30	0.21 - 1.66	B		
SSS	120	0.87 ± 0.25	0.35 - 1.85	AB		

		Fork Length (cm)				
Treatment	*n*	Mean ± S.D	Range	Significant differences	ANOVA (replicates)	ANOVA (treatments)
CCC	90	4.4 ± 0.4	3.2 - 5.2	AB	$F_{20,805} = 2.15$; $P < 0.01$*	$F_{7,833} = 2.67$; $P = 0.01$*
CCS	90	4.4 ± 0.2	3.0 - 5.0	A		
CSC	120	4.3 ± 0.4	3.2 - 5.4	AB		
CSS	120	4.3 ± 0.4	3.4 - 5.3	B		
SCC	90	4.3 ± 0.4	3.2 - 5.5	AB		
SCS	90	4.4 ± 0.4	3.6 - 5.4	AB		
SSC	120	4.3 ± 0.5	2.7 - 5.3	B		
SSS	120	4.3 ± 0.4	3.4 - 5.5	AB		

Treatment	n	*Body Condition* Mean ± S.D	Range	Significant differences	ANOVA (replicates)	ANOVA (treatment)
CCC	90	0.004 ± 0.027	-0.073 - 0.075	A	$F_{20,805}$ = 2.89; P < 0 .01*	$F_{7,833}$ = 1.44; P = 0.19
CCS	90	0.003 ± 0.045	-0.054 - 0.363	A		
CSC	120	0.002 ± 0.028	-0.062 - 0.136	A		
CSS	120	0.002 ± 0.032	-0.066 - 0.218	A		
SCC	90	-0.002 ± 0.040	-0.223 - 0.081	A		
SCS	90	-0.007 ± 0.023	-0.056 - 0.081	A		
SSC	120	0.000 ± 0.035	-0.096 - 0.160	A		
SSS	120	-0.003 ± 0.029	-0.100 - 0.123	A		

Note: All measurements were log transformed before analysis. Asterisks (*) indicate significant results a the $p < 0.05$ level.

Table 3. Recapture percentages (PIT and VIE tagged fish) and mass standardized growth rates (PIT tagged fish only) of *Salmo salar* released into Quaker Brook, New Brunswick for two sampling periods. Survival data for Sept-October 2011 also includes the 78 PIT tagged fish that were caught in May 2012 but not in October 2011, i.e. they must have been alive in October 2011 but were not caught. *F* and *P* values are displayed for one-way ANOVAs comparing treatment means for growth rate. Tukey's HSD post-hoc test was performed to compare multiple growth rate means of treatment groups. Levels not connected by the same letter are significantly different. * indicates treatment groups with the highest survival (recapture) numbers as calculated from a Generalized Linear Model Chi Square test.

Initial sampling period September 2011 - October 2011 (28 days in wild)

				Mass Standardized Growth Rate		
Treatment	# Released	Recapture %	Chi Square (d.f. = 7)	Mean ± S.D	Range	ANOVA
Comp - Simp - Simp	273	18.7*	$X^2 = 17.2$, p = 0.02	1.96 ± 1.04^{A}	0.30 - 5.49	$F_{7,253} = 21.0$; $P < 0.01$
Simp - Simp - Simp	285	12.3		1.77 ± 0.80^{A}	0.75 - 4.44	
Simp - Comp - Comp	282	12.8		1.81 ± 0.50^{A}	0.97 - 2.87	
Comp - Simp - Comp	285	9.1		1.71 ± 0.64^{A}	0.78 - 2.78	
Simp - Simp - Comp	277	11.9		0.79 ± 0.41^{B}	0.35 - 1.75	
Comp - Comp - Comp	263	12.5		0.77 ± 0.40^{B}	0.20 - 1.74	
Simp - Comp - Simp	262	18.3*		0.39 ± 0.70^{B}	-1.54 - 2.26	
Comp - Comp - Simp	266	12.4		0.59 ± 0.46^{B}	-0.40 - 1.39	

Overwinter period September 2011 - May 2012 (260 days in wild)

				Mass Standardized Growth Rate		
Treatment	# Released	Recapture %	Chi Square (d.f. = 7)	Mean ± S.D	Range	ANOVA
Comp - Simp - Simp	273	12.8*	$X^2 = 15.4$, p = 0.03	0.54 ± 0.08^{A}	0.43 - 0.71	$F_{7,168} = 6.2$; $P < 0.01$
Simp - Simp - Simp	285	8.1		0.53 ± 0.11^{A}	0.32 - 0.72	
Comp - Simp - Comp	285	5.6		0.53 ± 0.03^{AB}	0.45 - 0.67	
Simp - Comp - Comp	282	10.6		0.54 ± 0.07^{A}	0.41 - 0.70	
Simp - Simp - Comp	277	5.8		0.45 ± 0.10^{ABC}	0.35 - 0.73	
Comp - Comp - Comp	263	5.7		0.43 ± 0.11^{ABC}	0.31 - 0.61	
Simp - Comp - Simp	262	8.0		0.39 ± 0.10^{BC}	0.29 - 0.58	
Comp - Comp - Simp	266	7.5		0.37 ± 0.07^{C}	0.22 - 0.50	

Table 4. Chi-square results when comparing recapture rates between treatment groups at the first sampling event (28 d after release). Significant differences between groups (at the *p* < 0.05 level) are indicated by asterisks (*) and larger, bold font.

	CCC	CCS	CSC	CSS	SSS	SSC	SCS	SSC
CCC	x	x	x	x	x	x	x	x
CCS	n=513 df=1,511 x^2=0.06 p=0.80	x	x	x	x	x	x	x
CSC	n = 547 df=1,545 x^2=2.18 p=0.14	n=534 df=1,532 x^2=2.95 p=0.09	x	x	x	x	x	x
CSS	**n=539 df=1,537 x^2=4.06 p=0.04***	n=526 df=1,524 x^2=3.00 p=0.08	**n=560 df=1,558 x^2=12.54 p=0.00***	x	x	x	x	x
SSS	n=546 df=1,544 x^2=0.28 p=0.60	n=536 df=1,534 x^2=0.11 p=0.74	n=570 df=1,568 x^2=2.05 p=0.15	**n=562 df=1,560 x^2=4.56 p=0.03***	x	x	x	x
SSC	n=541 df=1,539 x^2=0.08 p=0.78	n=528 df=1,526 x^2=0.28 p=0.60	n=562 df=1,560 x^2=1.48 p=0.22	**n=554 df=1,552 x^2=5.40 p=0.02***	n=564 df=1,562 x^2=0.04 p=0.84	x	x	x
SCS	n=525 df=1,523 x^2=2.53 p=0.11	n=512 df=1,510 x^2=1.74 p=0.19	**n=546 df=1,544 x^2=9.56 p=0.00***	n=538 df=1,536 x^2=0.16 p=0.69	n=548 df=1,546 x^2=2.89 p=0.09	n=540 df=1,538 x^2=3.57 p=0.06	x	x
SCC	n=546 df=1,544 x^2=0.28 p=0.60	n=533 df=1,531 x^2=0.07 p=0.79	**n=567 df=1,565 x^2=4.17 p=0.04***	n=559 df=1,557 x^2=2.31 p=0.13	n=569 df=1,567 x^2=0.38 p=0.54	n=561 df=1,559 x^2=0.67 p=0.41	n=545 df=1,543 x^2=1.19 p=0.27	x

Table 5. Chi-square results when comparing recapture rates between treatment groups at second sampling event (over-winter ~ 260 d after release). Significant differences between groups (at the *p* < 0.05 level) are indicated by asterisks (*) and larger, bold font.

	CCC	CCS	CSC	CSS	SSS	SSC	SCS	SSC
CCC	x	x	x	x	x	x	x	x
CCS	n=513 df=1,512 x^2=1.07 p=0.30	x	x	x	x	x	x	x
CSC	n=547 df=1,545 x^2=0.00 p=0.97	n=534 df=1,532 x^2=1.18 p=0.28	x	x	x	x	x	x
CSS	**n=539 df=1,537 x^2=8.01 p=0.00***	n=526 df=1,524 x^2=3.11 p=0.08	**n=560 df=1,558 x^2=8.57 p=0.00***	x	x	x	x	x
SSS	n=549 df=1,547 x^2=1.17 p=0.28	n=536 df=1,534 x^2=0.00 p=0.99	n=570 df=1,568 x^2=1.30 p=0.25	n=562 df=1,560 x^2=3.28 p=0.07	x	x	x	x
SSC	n=541 df=1,539 x^2=0.00 p=0.98	n=528 df=1,526 x^2=1.04 p=0.31	n=562 df=1,560 x^2=0.004 p=0.95	**n=554 df=1,552 x^2=8.12 p=0.00***	n=564 df=1,562 x^2=1.15 p=0.28	x	x	x
SCS	n=525 df=1,523 x^2=1.10 p=0.29	n=512 df=1,510 x^2=0.00 p=0.99	n=546 df=1,544 x^2=1.22 p=0.27	n=538 df=1,536 x^2=3.17 p=0.07	n=548 df=1,546 x^2=0.00 p=0.99	n=540 df=1,538 x^2=1.08 p=0.30	x	x
SCC	**n=546 df=1,544 x^2=3.87 p=0.04***	n=533 df=1,531 x^2=0.81 p=0.37	**n=567 df=1,565 x^2=4.18 p=0.04***	n=559 df=1,557 x^2=0.87 p=0.37	n=569 df=1,567 x^2=0.83 p=0.36	**n=561 df=1,559 x^2=3.89 p=0.04***	n=545 df=1,543 x^2=0.82 p=0.37	x

Table 6. Initial length, weight and body condition of PIT tagged fish that were recaptured and fish that were not recaptured during May 2012 sampling period in Quaker Brook. One-way ANOVA models for weight and condition were non-significant ($F_{1,1364}$ = 2.7, p = 0.1 and $F_{1,1364}$ = 0.7, p = 0.4, respectively) and marginally non significant for length ($F_{1,1364}$ = 3.8, p = 0.05).

		Length (cm)	*Weight* (g)	*Condition*
Recaptured	*n*	Mean ± S.D	Mean ± S.D	Mean ± S.D
Yes	87	5.1 ± 0.4	1.7 ± 0.5	0.00 ± 0.03
No	1297	5.2 ± 0.4	1.7 ± 0.5	0.00 ± 0.03

Table 7. Final length, weight, and condition of PIT tagged fish recaptured in Quaker Brook during a sampling period in May 2012 (overwinter). One-way ANOVA models and Tukey's HSD post-hoc tests revealed no significant differences between treatment groups for all measures (all $p > 0.3$).

		Length (cm)	*Weight* (g)	*Condition*
Treatment	*n*	Mean ± S.D	Mean ± S.D	Mean ± S.D
Comp - Simp - Simp	15	8.9 ± 0.6	9.1 ± 2.2	-0.01 + 0.03
Simp - Simp - Simp	10	8.7 ± 0.6	8.8 ± 1.6	0.01 ± 0.02
Comp - Simp - Comp	7	9.1 ± 0.6	9.8 ± 1.8	0.00 ± 0.02
Simp - Comp - Comp	15	8.7 ± 0.7	8.7 ± 1.9	0.00 ± 0.04
Simp - Simp - Comp	10	8.9 ± 0.7	9.1 ± 2.2	-0.01 + 0.02
Comp - Comp - Comp	10	9.1 ± 0.7	9.7 ± 1.9	0.01 ± 0.03
Simp - Comp - Simp	10	8.7 ± 0.7	8.7 ± 2.3	0.00 ± 0.03
Comp - Comp - Simp	10	9.1 ± 0.9	10.2 ± 2.9	0.02 ± 0.02

Table 8. Total fish recaptured (PIT and VIE) and mass standardized growth rate (PIT only) of *Salmo salar* after roughly 260 d (overwinter) of rearing in semi-natural stream channels at Mactaquac Biodiversity Centre, New Brunswick. *F* and *P* values are displayed for one-way ANOVA test comparing treatment means for growth rate. Tukey's HSD post-hoc test was performed to compare multiple growth rate means of treatment groups. Levels not connected by the same letter are significantly different.

Overwinter period September 2011 - May 2012					
	Recapture		*Mass Standardized Growth Rate*		
Treatment	*n*	Chi Square (d.f. = 7)	Mean ± S.D	Range	ANOVA
Comp - Simp - Simp	79	$X^2 = 7.9$, p = 0.33	0.37 ± 0.13^{A}	0.11 - 0.92	$F_{7,613} = 21.4$; $P < 0.01$
Simp - Simp - Simp	84		0.38 ± 0.11^{A}	0.14 - 0.69	
Comp - Simp - Comp	77		0.37 ± 0.10^{A}	0.21 - 0.67	
Simp - Comp - Comp	73		0.37 ± 0.14^{A}	0.20 - 1.00	
Simp - Simp - Comp	73		0.26 ± 0.07^{B}	0.10 - 0.44	
Comp - Comp - Comp	79		0.27 ± 0.08^{B}	0.13 - 0.43	
Simp - Comp - Simp	78		0.23 ± 0.07^{B}	0.06 - 0.40	
Comp - Comp - Simp	78		0.24 ± 0.08^{B}	-0.18 - 0.41	

Table 9. Initial length, weight and body condition of PIT tagged fish that were recaptured and those that were not during May 2012 sampling period in semi-natural stream channels at MBC. One-way ANOVA models for weight and length were significant ($F_{1,448}$ = 5.2, p = 0.02 and $F_{1,448}$ = 6.6, p = 0.01, respectively) and not significant for condition ($F_{1,448}$ = 0.2, p = 0.6).

		Length (cm)*	*Weight* (g)*	*Condition*
Recaptured	*n*	Mean ± S.D	Mean ± S.D	Mean ± S.D
Yes	378	5.2 ± 0.4	1.7 ± 0.4	0.00 ± 0.03
No	72	5.3 ± 0.4	1.8 ± 0.5	0.00 ± 0.04

Table 10. Final length, weight, and condition of PIT tagged fish recaptured in semi-natural streams during the sampling period in May 2012 (overwinter). One-way ANOVA models and Tukey's HSD post-hoc tests revealed no significant differences between treatment groups for all measures (all $p > 0.7$).

		Length (cm)	*Weight* (g)	*Condition*
Treatment	*n*	Mean ± S.D	Mean ± S.D	Mean ± S.D
Comp - Simp - Simp	50	7.6 ± 1.0	4.5 ± 1.5	0.00 ± 0.06
Simp - Simp - Simp	52	7.6 ± 0.9	4.7 ± 1.7	0.00 ± 0.04
Comp - Simp - Comp	46	7.4 ± 1.0	4.4 ± 1.9	0.00 ± 0.04
Simp - Comp - Comp	43	7.6 ± 0.9	4.7 ± 1.7	0.00 ± 0.04
Simp - Simp - Comp	43	7.5 ± 0.9	4.6 ± 1.8	0.00 ± 0.05
Comp - Comp - Comp	49	7.5 ± 1.0	4.6 ± 2.0	0.01 ± 0.05
Simp - Comp - Simp	51	7.7 ± 0.9	4.7 ± 1.8	0.00 ± 0.04
Comp - Comp - Simp	48	7.4 ± 0.9	4.5 ± 1.8	0.00 ± 0.04

Figure Legends

Figure 1. Locations of Tobique River (origin river of fish used in this study), Quaker Brook wild release site, and Mactaquac Biodiversity Centre (MBC, inset).

Figure 2. Diagram of 1 m circular rearing tank and a photo of simple (left) and complex (right) rearing tanks used to rear juvenile Atlantic salmon (*Salmo salar*) from emergence through 60 d post-emergence.

Figure 3. Diagram of rearing paths from emergence through Stage 2.

Figure 4. Overhead diagram of semi-natural stream channels at the Mactaquac Biodiversity Centre. The total stream lengths were 31 m. The streambed was covered with gravel and cobble substrate, water velocities ranged from 1 to 72 cm • s^{-1}, and depths ranged from 12 to 60 cm (depth average = 35 cm). Therefore, an average of 87 m^2 of habitat was present in the stream channels.

Figure 5. Weight of Atlantic salmon fry throughout hatchery rearing period; Emergence, Stage 1 (30 d post-emergence), and Stage 2 (60 d post-emergence). Rearing environment is indicated as complex (C) or simple (S) and arrows represent movement from one rearing environment to the next from emergence through Stage 2 (also see Figure 3). Box plots represent the sample minimum, maximum, upper and lower quartile, and median.

Figure 1

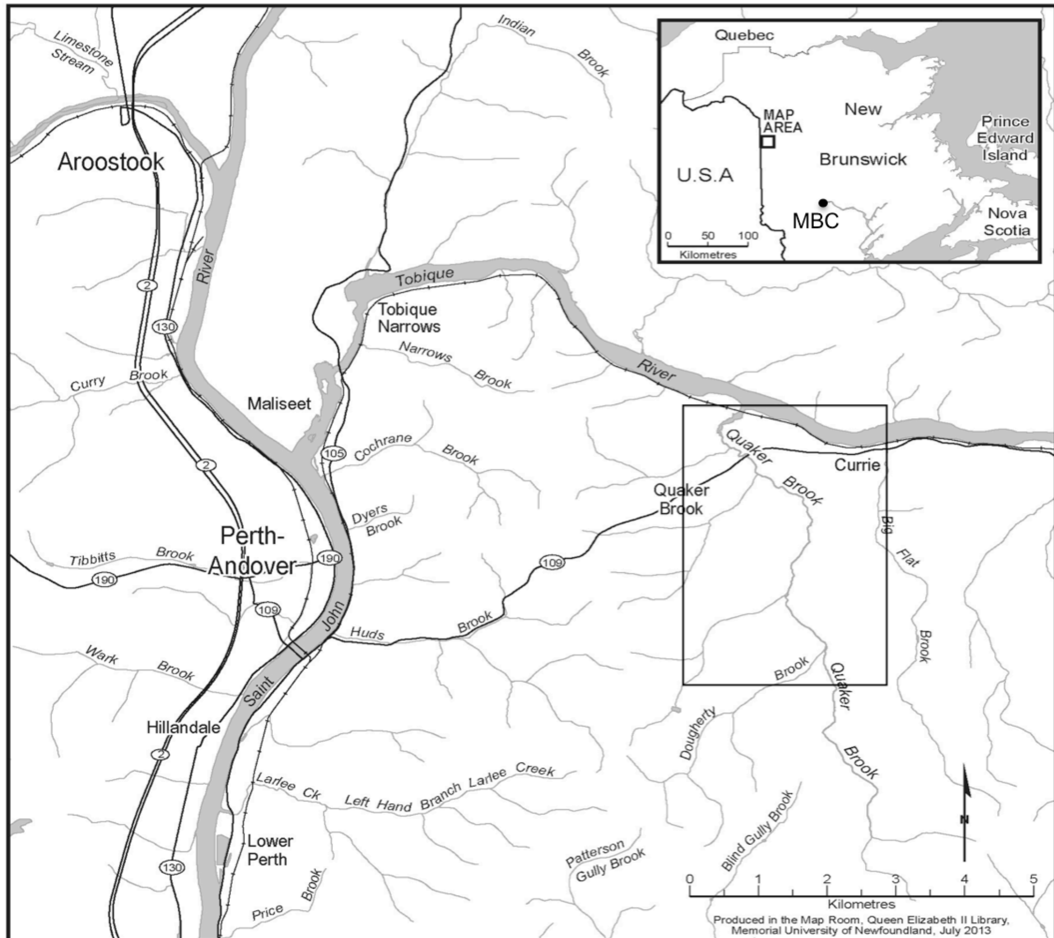


Figure 2

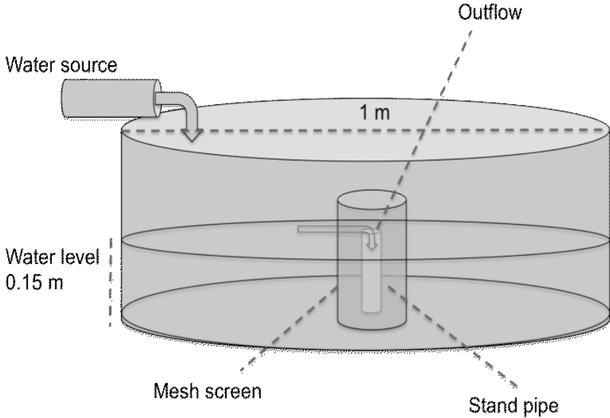


Figure 3

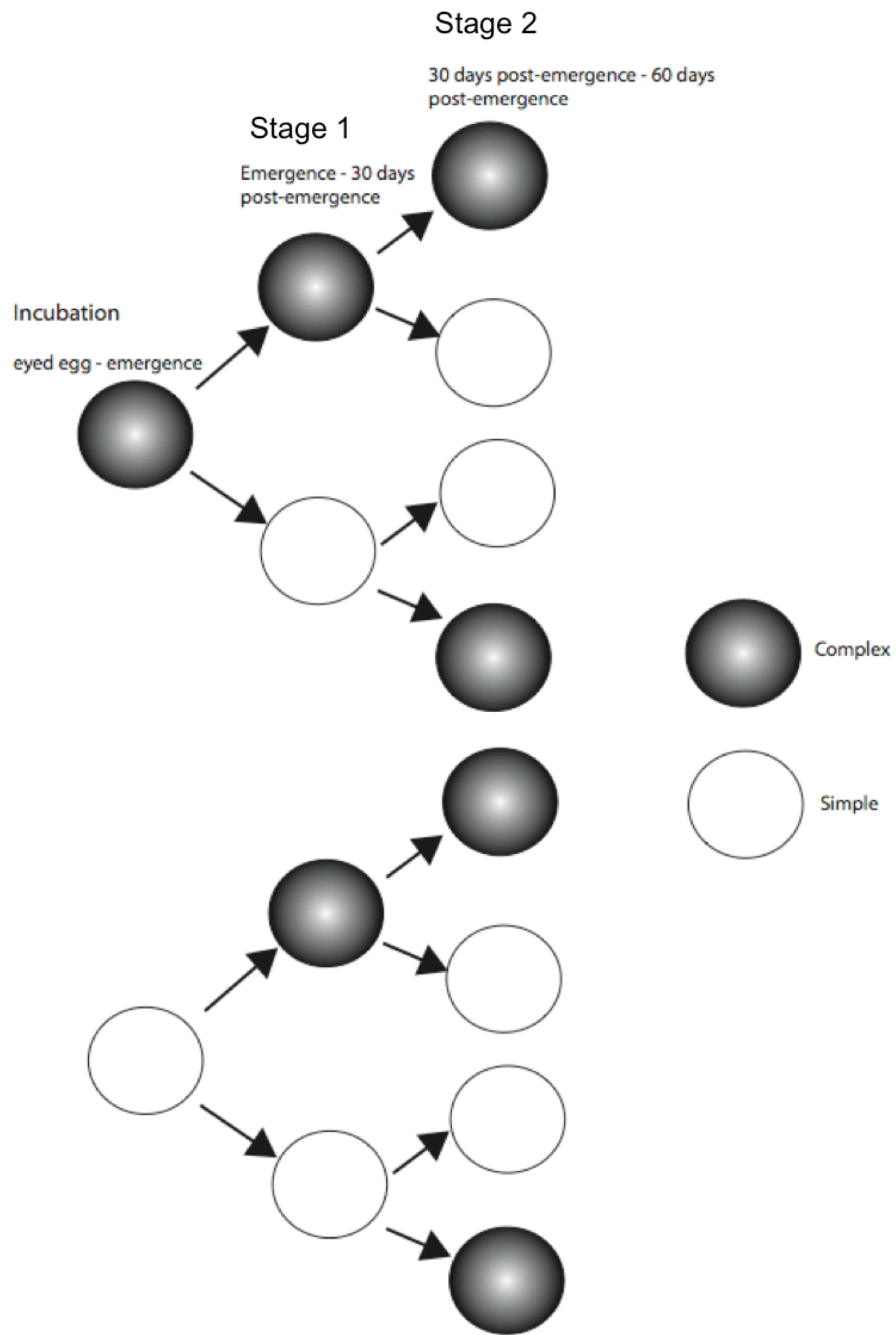


Figure 4

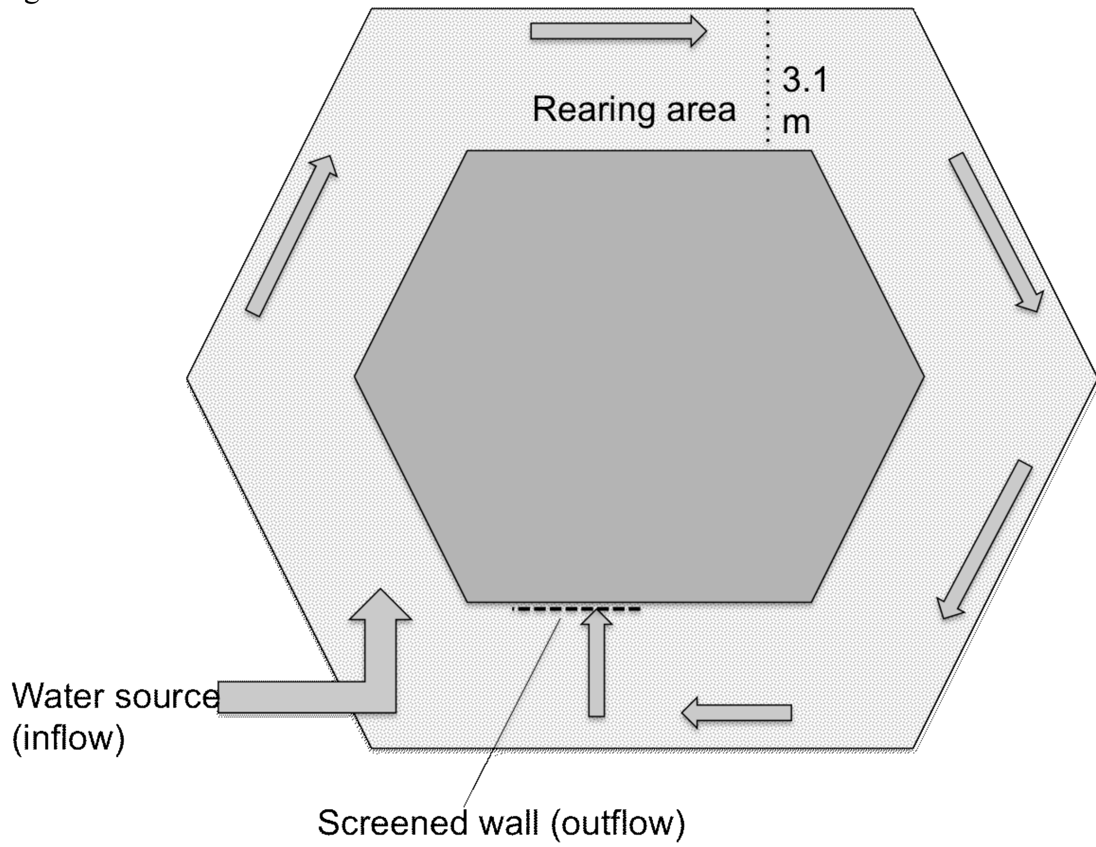
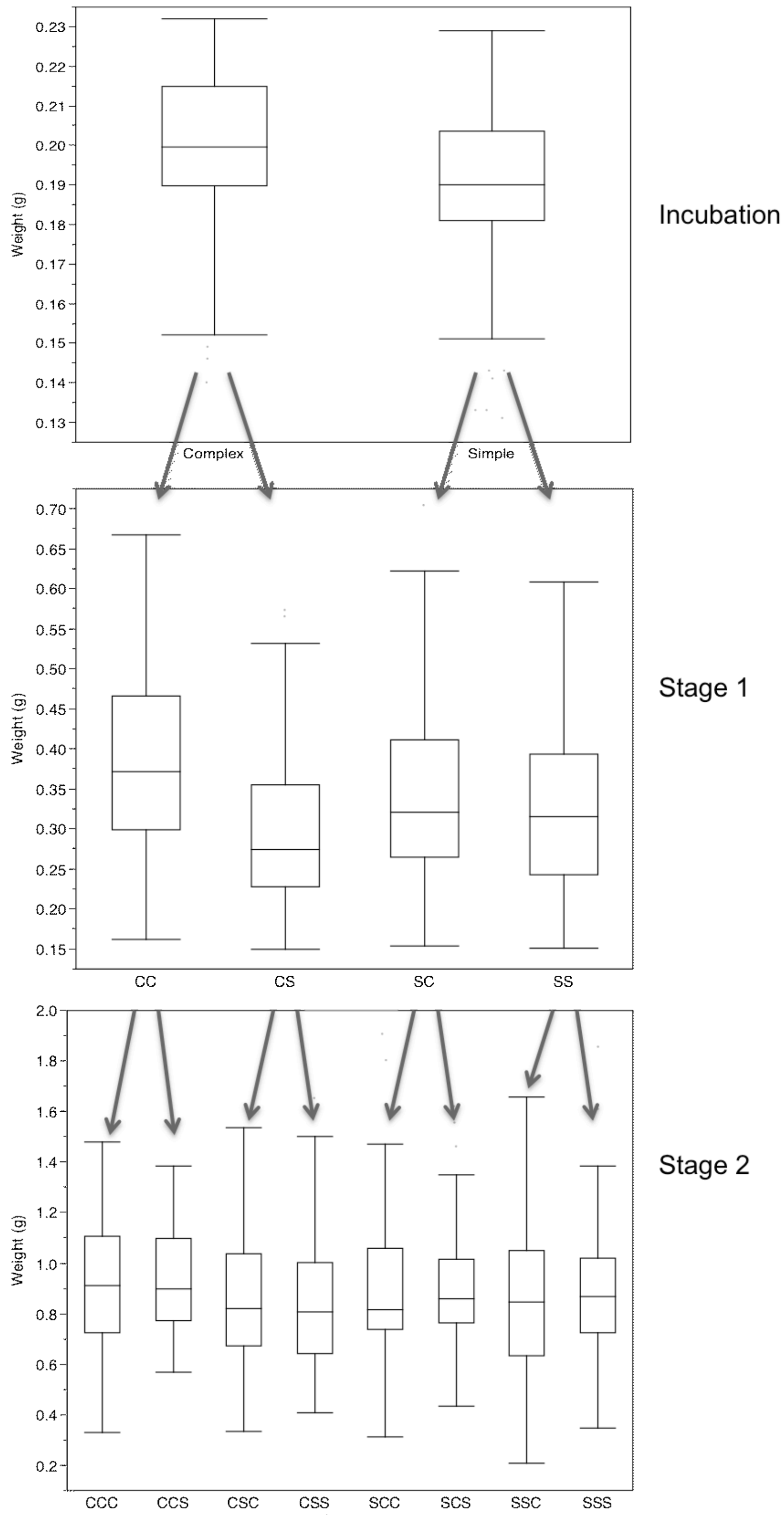


Figure 5



Chapter 4. Conclusion

Phenotypic plasticity is the ability of an organism to express two or more forms of a particular trait in response to environmental conditions (West-Eberhard 1989, 2003, Watters and Meehan 2007). Atlantic salmon are a model species for studying phenotypic plasticity because they must cope with a wide range of environmental conditions throughout their life (Aas et al. 2010 – Atlantic Salmon Biology, Hutchings 2011). For restocking, salmon are reared in facilities that are drastically different than wild environments and selective forces within fish-rearing facilities have led to phenotypic and genetic divergence of captive-reared fish from their wild counterparts (Fleming and Einum 1997). Consequences of such rearing practices, such as poor performance in wild environments, contribute to the failure of restocking programs to meet their goals (Hindar et al. 1991, Maynard et al. 2004, Le Vay et al. 2007, Jonsson and Jonsson 2009). Since salmon respond to environmental influences, recent efforts have attempted to mitigate this problem by manipulating conditions in fish-rearing facilities to promote the expression of phenotypic traits that may be more favourable in nature. The goal of this thesis was to investigate the influence of an environmental factor, habitat complexity, on phenotypic expression and performance in wild and semi-natural environments of juvenile Atlantic salmon (*Salmo salar*). In this conclusion, I review the key findings of each chapter and consider areas for future research.

In Chapter Two, I have attempted to better understand the role of habitat complexity on phenotypic development of juvenile Atlantic salmon at the emergence stage (pre-exogenous feeding). The results presented here suggest that adding gravel

during incubation can have a significant influence on phenotypic traits, including weight, body condition, and behaviour. I did not observe differences in brain volume or sub-structure volume (telencephalon, olfactory bulbs) between fish incubated with or without gravel. These results are contrary to some recent studies, however inconsistent results across juvenile salmon brain volume studies are beginning to surface. For example, Kihslinger and Nevitt (2006) found larger 'relative' cerebellar volumes of juvenile steelhead (*Oncorhynchus mykiss*) incubated with gravel and Näslund et al. (2012) found all measured regions to be larger in Atlantic salmon incubated in 'complex' environments, i.e. whole brain and sub-regions (telencephalon, cerebellum, olfactory bulb, optic tectum). In contrast, Kotrschal et al. (2012) observed larger brains in conventional hatchery-reared fish when compared to fish reared in "semi-natural" streams. Because methodologies used in measuring brain size varied across these studies, caution should be exercised in comparing results (reviewed by Healy and Rowe 2007). However, the results presented here coupled with those from previous studies could suggest that the relationship between brain volume and rearing environment in juvenile salmon is far more complex than originally thought and still requires significant investigation (discussed more below).

Fish that incubated in gravel were heavier, in better condition, fed on novel prey more readily and acted differently after a simulated predator attack when compared to fish incubated without gravel. These effects were most likely environmentally induced, since fertilized eggs from multiple males and females were pooled together (mixed) and randomly distributed to incubation environments. In other words, each incubation replicate held a random mixture of fertilized eggs from different parents. Observed

phenotypic differences amongst incubation groups likely contributed to greater growth and survival of gravel-incubated fish in semi-natural stream channels. Differences observed in behaviour amongst fish from the two incubation environments could suggest that neural processes of juvenile salmon may be influenced by habitat complexity in a way that is not reflected in brain volume. Most recently, Salvanes et al. (2013) found that adding structure to rearing tanks influenced gene expression and cognitive abilities of juvenile Atlantic salmon. Previous research has revealed that certain characteristics of the brain which can influence an animal's behaviour, such as neuron density and cell proliferation, can be influenced by environmental factors but may not be reflected in the overall size of an animal's brain (Mohammed et al. 2002, Lema et al. 2005, review by Healy and Rowe 2007). Therefore, I suggest that future studies consider measuring traits such as neuron density, brain cell proliferation, or gene expression to unravel the influence of habitat complexity on neural development in juvenile salmon (as reviewed by Ebbesson and Braithwaite 2012).

The results presented in Chapter Two add novel contributions to research investigating the influence of incubation environment on phenotypic development of juvenile Atlantic salmon at emergence. If employing a restocking strategy of releasing fish into the wild at emergence, the results presented here suggest that incubating fish at a hatchery without gravel could put fish at a disadvantage in terms of size, feeding activity, and behaviour (in response to a predator) when releasing them into the wild.

In Chapter Three, I have attempted to understand whether adding structural complexity to captive rearing environments for the first 60 days of exogenous feeding would influence growth and body condition and enhance post-release performance of

juvenile Atlantic salmon. Previous studies have attempted to investigate this topic, however my experimental design was unique in that it could identify if timing or duration of timing of exposure to habitat complexity would influence post-release performance, in terms of growth and survival. From an applied standpoint, this design is important because it could identify more labour or cost-effective methods of rearing fish in tanks with added structure for enhancing post-release performance.

During the captive rearing period, I detected body size and condition variation amongst treatment groups. These results suggest that structural complexity influences body size and condition in a hatchery setting. I detected differences among all treatment groups at emergence and after 30 days of exogenous feeding. At emergence, it is likely that gravel-incubated fish were able to utilize their yolk-sac energy reserves more efficiently than fish incubated without gravel, resulting in larger size (Hansen et al. 1985, Bamberger 2009). This is ecologically relevant because larger size at this point in early ontogeny is important with respect to avoiding size-related mortality, for example by exceeding gape-limitations of predators (Sogard 1997).

Fish that remained in complex environments for 30 days following emergence maintained their size advantage over other rearing treatment groups. This observation was likely facilitated by the size advantage of gravel-incubated fish at emergence. It is probable that the added structure also contributed to size differences, as it provided microhabitats for resting and metabolizing. Following the next growth period, the effects of adding structure to tanks on body size generally faded and at this point there were no differences in body condition. It is possible that density played a role in this ‘leveling’ out of size and body condition amongst treatment groups. The density of fish in rearing tanks

was reduced in half for the final captive-rearing period (due to the reciprocal-nature of the experiment). Reducing densities of fish in rearing tanks can minimize the effects of crowding, which have been observed to inflict negative effects on fish health, such as fin deterioration, and growth (Wagner et al. 1996, 1997, Brockmark et al. 2007). If poor conditions were minimized during the last rearing period, fish may have expressed a compensatory growth response in more favourable rearing conditions (Ali et al. 2003).

I detected a difference in survival amongst treatment groups in the wild but not in semi-natural stream channels. The differences in survival observed in the wild are difficult to interpret. This is due to the fact that groups that expressed higher survival overlapped in the duration of exposure and timing of exposure to complex rearing. Thus, it is unclear as to how habitat complexity influenced the findings in this study. These results are somewhat different from initial prediction that adding structural complexity to rearing tanks would enhance post release survival. I predicted that fish reared in complex environments for the entire hatchery-rearing period would have higher survival than other treatment groups. Studies by Brockmark et al. (2007) and Tatara et al. (2009) did not find clear effects of structural complexity on post-release performance of juvenile Atlantic salmon or steelhead, respectively. However, Tipping (1998) observed enhanced return rates of sea-run cutthroat trout reared as juveniles in gravel bottom ponds. Additionally, cutthroat trout that reared in these ponds for longer periods of time had enhanced return rates when compared to fish reared for less time in the ponds (Tipping 2001). My results coupled with those from previous studies highlight the need for more research on the influence of adding structure to rearing tanks on post-release performance to gain a better understanding on this topic.

We observed significant differences in specific growth rates between treatment groups in both semi-natural and wild environments. Groups with higher growth rates in nature also had higher growth rates in semi-natural stream channels over-winter, although growth rates were significantly higher in the wild. Higher growth rates observed in the wild amongst all fish are likely due to differences in prey availability and quality. Treatment groups that differed in growth rate overlapped in all aspects of timing and duration of timing of exposure to habitat complexity during captive rearing. Unfortunately, this makes it very difficult to suggest that habitat complexity during the captive-rearing period influenced post-release growth, as no clear pattern was evident. I did find that treatment groups with smaller fish at the time of release expressed higher specific growth rates compared to groups with larger fish at release. This finding could be a result of compensatory growth. Compensatory growth is a phase of accelerated growth following a period of growth suppression and it is often observed in salmon (Mortensen and Damsgard 1993, Nicieza and Metcalfe 1997, Ali et al. 2003). The fish that were smaller at release likely were not as successful under the captive rearing conditions used here, and needed to compensate for this when released into nature if they were to survive over winter.

Overall, the results presented in this thesis provide evidence that structural complexity in a captive setting can have a significant influence on phenotypic development of juvenile Atlantic salmon. At emergence, these differences in phenotype could be correlated with performance in a semi-natural environment. There is no evidence presented in this thesis suggesting an impact of structural complexity in a captive setting on juvenile Atlantic salmon brain volume at the emergent life cycle stage. Coupled with

previous studies, this highlights the need for further research on the influence of structural complexity on neural development beyond the scope of brain volume. From an applied standpoint, results from this thesis suggest that fish incubated without gravel may be at a disadvantage when released into nature. It could be noted that releasing fish into the wild from captive rearing earlier in ontogeny, such as at emergence, could reduce the chance for the development of maladaptive traits often observed in captivity. In addition, we found differences in survival between treatment groups in the wild. However these differences are difficult to interpret due to the fact that groups with higher survival overlapped with those with lower survival in duration and timing of exposure to complex rearing.

Atlantic salmon are valued in many ways and from a biologist’s (and catch-and-release fly-fisherman’s) perspective, more wild salmon in a river is indicative of a healthy ecosystem. However, if they are locally extinct we must act to try and restore the species and ecosystem that has been lost (likely as a result of anthropogenic disturbance). To work towards this goal, we must continue to put efforts into the development of captive-rearing strategies that promote the expression of phenotypic traits that are favourable in nature. Coupled with habitat restoration, perhaps we will discover a technique of captive-rearing-and-release that will help just enough to tilt the populations back toward an increasing trend. We could then discontinue captive-rearing and allow populations to re-establish themselves. The research presented in this thesis adds to this quest and provides opportunity for future research.

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Appendix A. Standard protocol for formalin tissue processing on automated vacuum infiltration tissue processor “TissueTek VIP”

# steps	Solution	Time, h	Temperature, °C	Pressure/ Vacuum	Agitation
1	10% Normal Buffered Formalin	0	37	on	on
2	10% Normal Buffered Formalin	0	37	on	on
3	70% Ethyl Alcohol	1	37	on	on
4	80% Ethyl Alcohol	1	37	on	on
5	95% Ethyl Alcohol	1	37	on	on
6	95% Ethyl Alcohol	1	37	on	on
7	100% Ethyl Alcohol	1	37	on	on
8	100% Ethyl Alcohol	1	37	on	on
9	100% Xylene	1	37	on	on
10	100% Xylene	1	37	on	on
11	Paraffin	1	60	on	on
12	Paraffin	1	60	on	on
13	Paraffin	1	60	on	on
14	Paraffin	1	60	on	on

Protocol for Hematoxylin and Eosin Staining

Procedure:

1. Deparaffinize sections, hydrate through graded alcohols to water.
2. Rinse in water 1 min.
3. Stain in Mayer's hematoxylin for 15 min.
4. Rinse in water.
5. Place in STWS for a few minutes until sections are blue.
6. Rinse in water for 5 minutes.
7. Stain in eosin 15 sec-2 min.
8. Dehydrate quickly through alcohols, clear in xylene and mount.

Results:

Nuclei: blue with some metachromasia

Cytoplasm: various shades of pink (identifying different tissue components)

Appendix B. Hatchery tank enrichment affect cortisol levels and shelter seeking in Atlantic salmon (*Salmo salar*)

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Abstract

Stocking programs using hatchery reared salmon are often implemented for augmenting natural populations. However, survival of these fish is often low compared to wild conspecifics, possibly due to genetic, physiological, and behavioural deficiencies. Here, we compared pre-smolt Atlantic salmon from three different environmental treatments (barren environment, plastic tube enrichment and plastic shredding enrichment) with regard to plasma cortisol levels, shelter seeking behaviour, and fin deterioration. Basal plasma cortisol levels were higher in barren-reared fish, indicating higher stress levels, while no differences were found in acute cortisol response after a 30 minute confinement test. Shelter seeking was higher in salmon reared in enriched tanks when tested alone, but not when tested in small groups. Barren-reared fish had higher levels of fin deterioration over winter, potentially due to higher aggression levels. These results suggest that enrichment can reduce the impact of stressors experienced in the hatchery, and thus increase fish welfare. Tank enrichment may also be used to produce salmon better adapted for the more complex environment encountered after release.

Keywords: Adaptive behaviour, Aquaculture, Environmental enrichment, Stocking, Stress

Introduction

Many Atlantic salmon (*Salmo salar* L.) populations have experienced severe declines across their natural range due to anthropogenic disturbances, including damming of rivers, deforestation, pollution, and overexploitation (Parrish et al. 1998, Fraser 2008, Piccolo et al. 2012). To compensate for these declines, hatchery-reared salmon have been stocked into the wild through supplementation and enhancement programs (Jonsson and Jonsson 2006). However, when compared to wild conspecifics, hatchery-reared salmonids typically experience lower survival in nature (e.g. Berg and Jorgensen 1991, Jonsson and Jonsson 2006, Kallio-Nyberg et al. 2011). The importance of focusing on survival instead of number of released fish has recently been stressed (e.g. California Hatchery Scientific Review Group 2012).

Poor survival of hatchery-reared fish in the wild can be attributed to a number of factors. The captive environment differs from the wild as habitat complexity is typically low, food is invariably abundant, diseases are treated, densities are high, predators are absent and human disturbance is often high (Huntingford 2004). Traits and deformities that would be disadvantageous in nature can arise in hatchery environments, e.g. maladaptive behaviour and fin erosion due to abrasion with the tank floor, infections and, especially, aggressive acts between individuals (Latremouille 2003, Huntingford 2004, Ashley 2007). Fish in hatcheries can thereby be subjected to rapid domestication selection resulting in adaptation to the captive environment, but also to relaxed selection pressures on traits favourable in nature, both of which could be detrimental for survival in the wild (Frankham et al 1986, Christie et al 2012).

In fish reared for conservational stocking, behaviour adapted for natural environments are critical for stocking effectiveness (Brown and Day 2002, Salvanes and Braithwaite 2006). Several actions have been suggested as solutions to reduce maladaptive behaviours, e.g. life-skills training, social learning protocols, acclimatisation at release, and environmental enrichment (reviewed by Brown and Day 2002). Recent studies on several fish species suggest that enriched captive environments can promote foraging abilities (Brown et al. 2003, Strand et al. 2010, Rodewald et al 2011) and behavioural flexibility (Braithwaite and Salvanes 2005). Additionally, it may also influence social interactions (Berejikian et al. 2000, Salvanes and Braithwaite 2005) and reduce anxiety (Maximino et al. 2010). In conventional barren hatchery tanks with high fish densities, juvenile salmon adopt shoaling behaviour which contrasts with their natural territorial behaviour. Adding shelter-structures to the rearing tanks could render other, more natural, behavioural strategies possible. In addition, the presence of shelters may also reduce the impact of disturbance and stressors in the hatchery environment (Barton and Iwama 1991).

Fish in controlled aquaculture facilities are subjected to several stressors, including human disturbance and aggressive interactions with conspecifics (Schreck 1982, Pickering 1993). In response to stressors fish coordinate physiological and behavioural responses, with the primary physiological response being the release of major stress hormones like corticosteroids and catecholamines (Wendelaar Bonga 1997). Cortisol, a corticosteroid released by the hypothalamic-pituitary-interrenal axis (HPI-axis), is frequently used as a marker for both acute and long term stress in fish (Barton 2002, Martínez-Porchas et al. 2009). Increased cortisol expression can lead to energetic costs

(Barton and Iwama 1991), and thus affect other energy demanding processes, such as growth (McCormick et al. 1998), immune function (Tort 2011), reproductive output (Campbell et al. 1994), and neurogenesis (Sørensen et al. 2011). Access to shelters can reduce metabolic demands in Atlantic salmon, which may be, at least in part, due to reduced stress (Millidine et al. 2006, Finstad et al 2007). For instance, brown trout (*Salmo trutta*) juveniles, which have similar social behaviours as Atlantic salmon, show lower aggression levels in complex environments where visual contact is reduced (Sundbaum and Näslund 1998, Höjesjö et al. 2004). Adding structures to visually isolate the fish may reduce overall stress levels and injuries through decreased social aggression, and also shield against other environmental disturbances.

In this study we investigate how adding structure in rearing tanks affect stress levels, aggressive attacks and shelter-seeking behaviour in under-yearling Atlantic salmon. The specific predictions tested were: 1) fish reared in tanks with artificial shelters maintain lower basal levels of plasma cortisol compared to fish reared in barren tanks; 2) fish from barren tanks have reduced hormonal response to an acute stressor, as repeated exposure to stressors in the hatchery may cause desensitisation or exhaustion of the HPI-axis (Wendelaar Bonga 1997); 3) structural enrichment reduce aggression as indicated by lower levels of dorsal fin deterioration (Abbott & Dill 1985); and 4) juveniles reared in enriched tanks would use shelter to a higher degree than fish from barren tanks. Descriptive data on body size and growth are presented as electronic supplementary information.

Materials and methods

Rearing conditions

First generation hatchery bred juvenile Atlantic salmon originating from the River Imsa stock (58° 54’N, 5° 57’E) were held at Ims Research Station (Norwegian Institute for Nature Research), Norway, for 31 weeks (September 29, 2010 – May 2, 2011) in 1 m^2 tanks under three different rearing conditions (Fig. 1): 1) standard barren hatchery environment, or enrichment via the addition of 2) plastic tubes (length = 10 - 20 cm; Ø = 4 cm), or 3) shredded black plastic bags (length = 60 cm). Enrichment structures covered approximately half the tank floor area and were bundled together in each tank to facilitate their removal. Each environmental condition was replicated twice with 80 underyearling salmons in each tank. All fish had been reared in standard barren tanks since the start of feeding and had a similar size distribution upon release into treatment tanks (Table S1 and Fig. S1, supplemental information). Tanks were supplied with fresh naturally tempered water from a nearby lake and commercial food pellets were given in excess from automatic feed dispensers (Ewos No 505, Ewos AS, Skårer, Norway). The light regime was 12:12 h (light:dark). Individual data on growth and fin deterioration was obtained from 60 individuals from each tank which were tagged with passive integrated transponders (PIT-tags), the rest of the fish had their adipose fin clipped for quick identification of non-tagged fish. All tanks were subjected to daily cleaning, which included water level reduction and brushing. In addition, enrichment structures were briefly lifted out of the tanks and cleaned two to three times per week.

23 weeks into the treatment (on March 10 - 11, 2011) subsamples of the fish from each tank were subjected to the cortisol measurements, stress test and shelter seeking trials.

Plasma cortisol

When examining basal levels of cortisol, six fish from each replicate tank were netted simultaneously. Any enrichment structures were quickly lifted out of the water while the fish was netted. All fish were killed immediately after netting by a sharp blow to the head and blood was sampled within the window of cortisol excretion, < 4 minutes (Gamperl et al. 1994). Basal level samples were taken during day-time when cortisol activity should be at relatively low levels (Thorpe et al. 1987).

To determine acute stress levels we used a standard 30 minute confinement test (Barton and Iwama 1991), which investigates behavioural and physiological responses to a stressor without inflicting physical harm to the fish. Previous studies in Atlantic salmon have shown an acute cortisol response can be reached within this time frame when severe stressors are applied (Einarsdóttir and Nilssen 1996, Iversen et al. 1998) and that this method can separate groups differing in HPI-axis reactivity (Kittilsen et al. 2009). Five fish from each replicate tank were netted at random and placed individually in narrow, well circulated boxes (10 × 15 cm) with a low water level (mean 4.45 cm; range 4 - 7 cm). Information regarding activity during confinement is found in the online supplemental material. Following confinement, fish were killed by a sharp blow to the head and the blood was sampled within four minutes.

All blood samples were taken by caudal venipuncture, using heparinised syringes. Thereafter, plasma was immediately separated by centrifuge and stored in -20°C for 2 - 3 days before being transported on dry ice to University of Gothenburg, Sweden, where it was kept at -80°C until analysis. Cortisol concentrations were measured in unextracted plasma by a radioimmunoassay following methods in Young (1986) and Sundh et al. (2011), with sheep-anti cortisol antibodies (Code: S020; Lot: 1014-180182; Guildhay Ltd., Guildford, Surrey, U.K.), using a β-counter (Wallac 1409 Liquid Scintillation Counter, Turku, Finland). Hydrocortisone-[1,2,6,7-^{3}H(N)] (NET 396, NEN Life Sciences Products, Inc., Boston, MA, USA) was used as tracer and cortisol standards were prepared from hydrocortisone (Sigma-Aldrich, St. Louis, MO, USA). Intra- and inter-assay coefficients of variation (CV) for cortisol assays have, based on previous measurements in our lab, been assessed to be 3.9% and 5.4% respectively (Sundh et al. 2011). The detection limit was 0.8 ng · ml^{-1}. One individual from each treatment in the basal cortisol sample and one individual from the acute sample (shredded plastic treatment) were withdrawn from the analyses due to low volumes of extracted plasma or complications during the assay.

Basal (BC) and acute (AC) plasma cortisol concentrations were analysed using linear mixed models with cortisol levels as the dependent variables. The model used was:

$$Response\ variable = Intercept + Treatment + Tank(Treatment) \quad (1)$$

with *Treatment* as a fixed factor and *Tank* nested within *Treatment* as a random effect block. The model was fitted using restricted maximum likelihood (REML). Homogeneity of variance was tested using Levene's test (BC: $p = 0.341$; AC: $p = 0.136$). Normality was tested using Shapiro-Wilks test ($p > 0.05$ for all tanks in both BC and AC). Bonferroni adjusted pairwise comparisons were made based on the estimated marginal means.

Sheltering behaviour

Sixteen test tanks (60 × 60 cm) were divided into a release section (section I) and a shelter section (section II) by metal mesh screens (mesh size 7 × 7 mm). Two openings (4

× 5 cm) were cut out in the screens at 10 cm from each of the tank walls (see test tank setup in Fig. 2). These openings were elevated 15 cm from the bottom so that fish had to actively swim upwards to find the way to the other side. Water level was kept at 25 cm in all tanks. Three plastic tubes (identical with the tubes in the second enrichment treatment) were placed in section II.

During the trials the positions of the fish in the tanks were recorded every ten minutes for one hour. A fish was considered sheltered when at least the anterior half of the fish was inside a tube.

Two experiments were performed in this setup where fish were tested either individually or in groups of three. Four rounds of trials were run for each experiment, adding up to sample sizes of ten fish or groups from each replicate tank. Each round lasted one hour and each fish was observed every ten minutes. Bias due to test tank effects was minimised by systematically varying the treatment and replicate tank tested.

Individual behaviour was analysed by assigning each fish a binomial score, “using shelter” or “not using shelter”, depending on whether the fish used shelter or not at any of the observations during the trial (i.e. all observations for a fish were combined to one score). We used a generalised linear mixed model (GLMM) for binomial data with a log link function to analyse the scores as dependent variables according to model (1).

Group behaviour was analysed based on the maximal proportion of fish in a tank sheltering at any of the observations during a trial. The statistical model used was a GLMM to fit a binomial regression with a log link function according to model (1).

Pairwise post-hoc comparisons were performed on the estimated marginal means using sequential Bonferroni adjustment.

Fin deterioration

Dorsal fin damage was scored from 1 to 3 for individual PIT-tagged fish with 1 = negligible damage, 2 = less than 50% of fin area eroded, and 3 = more than 50% of fin area eroded. Analyses were performed on the change in fin score (i.e. deterioration) between measurements (September 29, 2010; November 18, 2010; and May 2, 2011) using a generalised linear model with a multinomial probability distribution and a cumulative logit link function according to model (1), but with Tank(Treatment) as a fixed factor to detect tank effects from this specific experimental setup.

General notes

Water supplied during the experiments came from the hatchery's naturally tempered source (2°C). All statistical analyses were made in IBM SPSS Statistics 20 (SPSS, Inc., an IBM Company, Armonk, NY). Threshold for significance was $p = 0.05$; pairwise comparisons are presented as adjusted *p*-values.

Results

Plasma cortisol concentration

The basal plasma levels of cortisol differed significantly among treatments ($F_{2,30} = 11.675$, $p < 0.001$) with the fish from the simple environment having higher levels than groups from enriched environments ($p \leq 0.002$ for both pairwise comparisons, Fig. 3a). The two enrichments did not differ significantly.

The acute plasma levels of cortisol following the confinement test did not differ significantly ($F_{2,26} = 0.051$, $p = 0.950$; Fig. 3b).

Sheltering behaviour

The individually tested fish showed a significant treatment effect on sheltering behaviour ($F_{2,57} = 4.021$, $p = 0.023$; Fig. 4a). Pairwise comparisons showed that the fish reared in either of the enriched environments used shelters to a higher proportion than fish reared in barren tanks (barren vs. shredding: $p = 0.035$; barren vs. tubes: $p = 0.006$; shredding vs. tubes: $p = 0.521$).

The fish tested in groups of three showed no significant differences in sheltering behaviour (maximum proportion of fish sheltering at any time) ($F_{2,57} = 0.263$, $p = 0.769$; Fig. 4b).

Fin deterioration

Over the autumn (September to November) there was no apparent fin deterioration in any treatment (Wald $\chi^2 = 3.812$, df = 2, $p = 0.149$), but there was a trend for tank effects (overall: Wald $\chi^2 = 7.251$, df = 3, $p = 0.064$) originating from differences between the barren replicates and the shredding replicates ($p = 0.069$ and $p = 0.059$ respectively; tubes: $p = 0.370$). Between November and May there was a significant treatment effect where the barren treatment had a greater increase in fin deterioration (Wald $\chi^2 = 16.137$, df = 2, $p < 0.01$). There were also significant effects or trends in tank effects in all treatments (overall: Wald $\chi^2 = 12.096$, df = 3, $p = 0.064$; barren: $p = 0.083$; shredding: $p =$

0.045; tubes: p = 0.022). Box plots regarding fin variables are found in the electronic supplement (Fig. S3 and Fig. S4).

Discussion

Fish from barren tanks had on average two to three times higher basal levels of plasma cortisol than fish from the enrichment treatments. Such elevated basal cortisol levels could be an effect of intermittent and unpredictable stressors (Ladewig 2000). In fact, the measured basal cortisol concentrations of fish in the barren tanks were similar to the levels found when exposing Atlantic salmon juveniles to chronic stress (Fridell et al. 2007). It is possible that the presence of structures shields against intermittent external disturbance as well as conspecific aggression and potentially creates a lower-stress environment. Lower aggression among fish in enriched environment is indeed supported by lower degrees of dorsal fin deterioration between November and May. Two recent studies on zebrafish (*Danio rerio*), found either no enrichment effect on cortisol (Wilkes et al. 2012) or slightly elevated cortisol concentrations (von Krogh et al. 2010). This may be due to species specific responses to structural enrichments, but differences in time span, type of enrichment and experimental setting (laboratory) may have influenced the results too.

During visual inspections, we observed that the fish highly utilized the provided enrichment. Sheltering is a natural behaviour in salmon parr, especially at winter temperatures such as those observed during our measurements (Heggenes and Saltveit 1990). Thus, having the opportunity to perform naturally preferred behaviours might

reduce distress and increase welfare of hatchery fish, as it does in captive mammals (Carlstead et al. 1993, Boinski et al. 1999).

The fish in our study were subjected to ordinary activity in the hatchery and thus to typical disturbance levels experienced by hatchery-reared fish. In addition, there was unavoidable disturbance in enriched tanks during removal and cleaning of the enrichment structures. This could have been an additional stressor in the enriched tanks. However, our results suggest that presence of shelters in the rearing environment is beneficial overall.

Regarding sampling methodology, the sampled fish in each tank were netted in one sweep and immediately killed. With this in mind and given the low temperature, which would have made the cortisol response relatively slow, it was unlikely that there was any sampling bias due to procedure (Gamperl et al. 1994; Barton 2002).

In contrast to the basal plasma cortisol levels the acute cortisol response was similar between all environmental treatments. Levels were comparable to those commonly observed in salmonids after acute stress (Pickering and Pottinger 1989, Barton 2000, 2002), suggesting that the cortisol response was most likely not blunted in barren reared fish. Thus, it appears that there still is a capacity for primary stress response to critical stressors in all treatments.

Our study suggests that, in addition to the stress reducing effects, environmentally enriched rearing environments increases the shelter-seeking behaviour of hatchery salmon after release into a novel environment. Studies in both Atlantic cod and Atlantic salmon show that time spent sheltering can be increased by enriched rearing environments (Salvanes and Braithwaite 2005, Roberts et al. 2011). However, there were no significant

effects on the usage of woody structures by environmentally enriched steelhead trout (*Oncorhynchus mykiss*), after release into a quasi-natural stream (Berejikian et al. 2000). It is likely that there are substantial species and population differences in sheltering behaviour (Valdimarsson et al. 2000) and sheltering is also likely to depend on experience and context (Brown and Warburton 1997, Berejikian et al. 1999, Álvarez and Nicieza 2003). Increased shelter-seeking behaviour in our experimental setup, where fish had to actively swim to an adjacent compartment of the tank to reach shelters, was consistent with results showing increased exploratory behaviour in enrichment-reared steelhead trout (Lee and Berejikian 2008). Lack of differences in the group-trials may be due to a different context. While sheltering can reduce risk, it may also inhibit competitive behaviour for resources. In a group the predation risk is lower and the competition higher which could lead to higher levels of activity (e.g. by reduced sheltering) when fishes are in groups compared to when they are alone (Bohlin and Johnsson 2004).

The fitness of hatchery-reared salmonids released for wild stock enhancement is often low, which could depend on both genetic and phenotypic alterations in the captive environment (Brown and Day 2002). The release event is most likely a stressful event and previous studies on Pacific salmonid species have shown that acute stress reduces anti-predator behaviour for a short while (Olla et al. 1992, Mesa 1994). Hence, displaying active shelter seeking in the behavioural repertoire at release could be advantageous for the immediate survival of recently released fish in predator-rich environments (e.g. Warner et al. 1968, Aarestrup et al. 2005, Kekäläinen et al. 2008).

In conclusion, our main results suggest that environmental enrichment in the form presented here can reduce basal stress levels as indicated by plasma cortisol

concentrations and potentially provide hatchery-reared Atlantic salmon with a more advantageous anti-predator behaviour in the form of shelter seeking.

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Figure Legends

Fig. 1. Rearing environments and treatment tank order in the hatchery. B = Barren (barren tanks), T = Plastic tube enrichment, S = Plastic shredding enrichment. In the picture of the barren tank a net covering the tank is seen; the same kind of nets also covered the enriched tanks (removed in the pictures).

Fig. 2. Tank setup for the shelter seeking trials. To the left a model of the trial tank showing the position of the holes in the mesh screen and to the right a schematic top-view showing the position of the shelters. Section I was the release section and section II was the shelter section. Plastic tubes were used as shelters.

Fig. 3. Plasma cortisol concentration at: (a) basal levels, and (b) acute levels after confinement. B = fish from barren tanks, S = fish from plastic shredding enrichment, T = fish from plastic tube enrichment. Error bars show the 95 % Wald confidence interval. Asterisk denote a significant difference compared to the other groups ($p < 0.05$).

Fig. 4. Sheltering seeking propensity over one hour in novel environment. (a) Proportion of fish sheltering in the single fish trials. (b) Maximal proportion of fish sheltering at the same time in group trials. B = fish from barren treatment (barren tanks), S = fish from plastic shredding enrichment, T = fish from plastic tube enrichment. Error bars show the 95 % Wald confidence interval. Asterisk denote a significant difference compared to the other groups ($p < 0.05$).

FIGURE 1

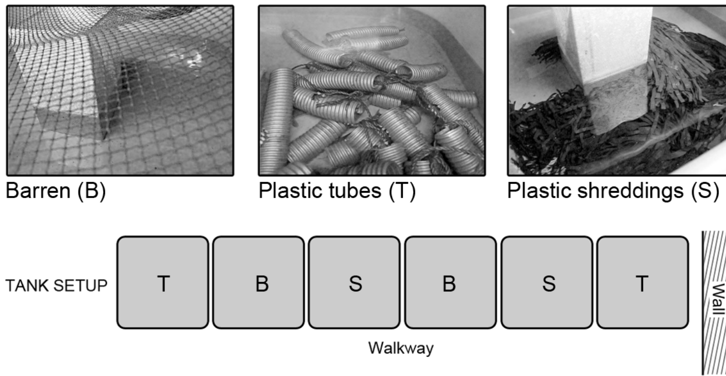

FIGURE 2

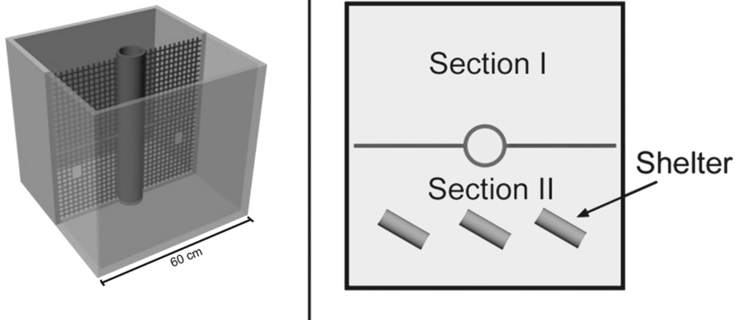

FIGURE 3

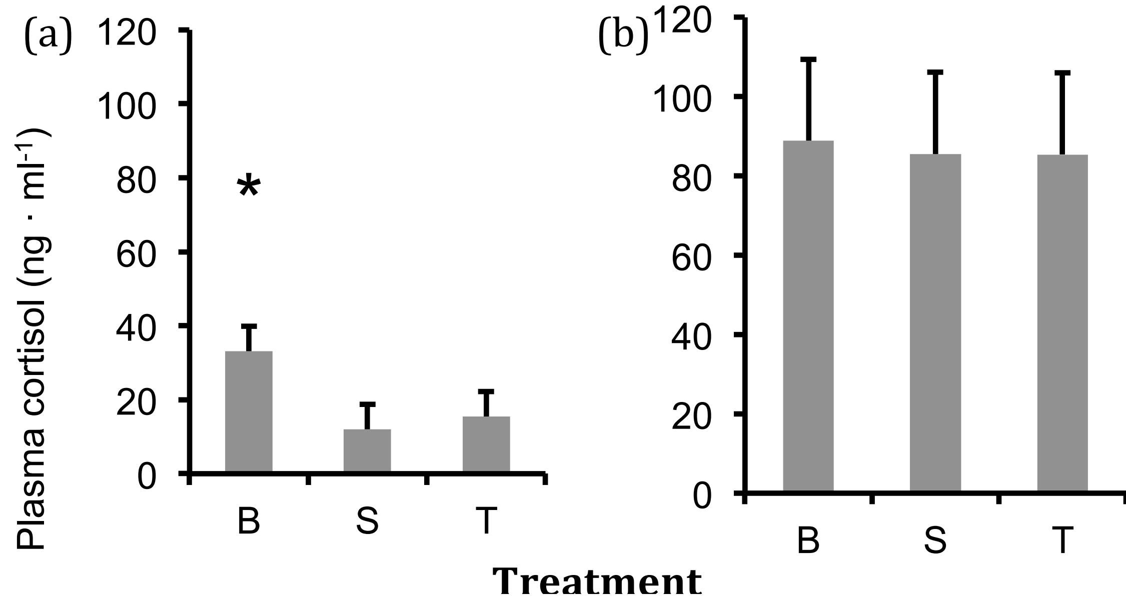

FIGURE 4