Born to be wild: effects of rearing density and environmental enrichment on stress, welfare and smolt migration in hatchery reared Atlantic salmon

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Born to be wild: effects of rearing density and environmental enrichment on stress, welfare and smolt migration in hatchery reared Atlantic salmon

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Abstract

Hatchery reared salmonids released into the wild generally have poor survivability compared to wild conspecifics. In order to assess potential hatchery rearing improvements, behavioral and physiological effects of reducing animal density and adding in-tank shelter were investigated. Atlantic salmon parr were placed in barren or shelter enriched tanks at high or low density up until release as smolts. A lowered density rendered positive effects on growth and intestinal barrier function and the combination of a lower density and shelter decreased conspecific aggression, as inferred by fin damage. Furthermore, while the presence of shelter decreased stress hormone levels following human disturbance it also decreased growth and smolt migration success, an effect particularly pronounced at high densities. Therefore, we suggest that this type of structural enrichment should be avoided for Atlantic salmon smolts held at high densities and conclude that a lowered animal density with or without shelter has the highest potential in producing a more resilient smolt for stocking.
Human impact, through overexploitation, habitat degradation and climate change are thought to be causing an historical sixth mass extinction (Barnosky et al. 2011). Therefore, supplementation and re-introduction programs are believed to be important future efforts to conserve biodiversity (Seddon et al. 2007; Barnosky et al. 2011). However, the survival and fitness of released animals are generally low and experimental data on the effects of the captive environment on phenotypic development and post-release performance are limited (Fischer and Lindenmayer 2000; Seddon et al. 2007). Atlantic salmon (Salmo salar L.) have experienced severe regression because of anthropogenic disturbances (Parrish et al. 1998; Fraser 2008) and captive bred juveniles are released to ensure viability of genetically distinct populations (Jonsson and Jonsson 2006). The observed low survival compared to wild salmon is suggested to stem from different experiences and selection pressures during early life stages (Jonsson and Jonsson 2006; Kallio-Nyberg et al. 2011; Hyvärinen and Rodewald 2013) and/or stress created by suboptimal rearing regimes (Jonsson and Jonsson 2006). Therefore, the identification of key factors for production of more wild-like and robust phenotypes is prioritized. (Brown and Day 2002; Thorstad et al. 2012).

Compared to nature, hatcheries represent a barren environment with high densities of fish, leading to little or no escape from conspecifics or other captive related stressors (Johnsson et al. 2014). Lowered density and in-tank structure could therefore represent two feasible modifications (Johnsson et al. 2014).

In salmonids, exposure to stress and suboptimal rearing regimes in the juvenile stage is known to negatively affect immune functions (Sundh et al. 2010) and to increase mortality after transfer to seawater (Fridell et al. 2007). While primary physiological responses to stressors, like the release
of stress hormones, are adaptive and mainly positive, they can result in negative secondary or tertiary effects on both behaviour (Gaikwad et al. 2011) and physiological mechanisms (Olsen et al. 2005; Niklasson et al. 2011). The intestine is a stress sensitive organ, and through a decreased epithelial integrity stress can cause pathogen entry, infection and death (Murray and Peeler 2005; Fridell et al. 2007). The integrity of the intestinal primary barrier is therefore used in this study as a secondary stress marker and a proxy for future disease resistance (Berg 1995; Sundh et al. 2010; Segner et al. 2012).

Previous studies on lowered density and structural enrichment have shown positive effects through e.g. decreased aggression (Brockmark et al. 2007; Näslund et al. 2013). Furthermore reduced density has been reported to result in improved anti-predator behaviour (Brockmark et al. 2010) and increased survival after release (Brockmark et al. 2010; Brockmark and Johnsson 2010), whereas in-tank shelter have resulted in lower basal cortisol levels, increased shelter seeking behaviour (Näslund et al. 2013), enhanced disease resistance (Karvonen et al. 2016) and improved smolt migration (Hyvärinen and Rodewald 2013). There are, however, studies where in-tank shelter show no or even negative effects on e.g. post-release performance (Berejikian et al. 1999; Brockmark et al. 2010; Näslund and Johnsson 2014) and possible interactions between altered density and increased structural complexity are still highly unexplored.

There is a lack of studies evaluating feasible improvements to captive conservation programs, studying stress and welfare indicators, together with behavioural and physiological performance and post-release success. The aim of this study was therefore to 1) investigate if reduced animal density and structural enrichment affect growth, stress hormone responses, shelter seeking behaviour and intestinal primary barrier functions and to 2) examine if these measures results in positive effects on smolt migration success. We hypothesize that by reducing density and adding
complexity to the hatchery tanks, the environment will better reflect the wild habitat and render positive effects on the produced phenotype and on post-release performance.

Materials and methods

Experimental fish & Treatment

In autumn 2011, 15 male and 30 female wild Atlantic salmon originating from the River Imsa, Norway (58°54’N, 5°57’E) were captured and artificially spawned at Ims Research Station (Norwegian Institute for Nature Research). The eggs and fry were reared in horizontal flow-through hatching trays at ambient temperature until moved to standard barren hatchery tanks upon start-feeding in May 2012.

On Oct 8, 2012 a total of 2400 fish were randomly divided between the four treatments, each with three 2 m² opaque grey plastic tanks, water level approximately 30 cm. A 2×2 factorial design was used; with two densities of fish; high, following local standard hatchery practice (150 ind × m⁻², weight density in May 2013: 14.4 kg × m⁻³) and low density (50 ind × m⁻², mass density in May 2013: 4.8 kg × m⁻³) in combination with barren or structurally enriched tanks. This created four treatment groups: High Density/No Shelter, High Density/Shelter, Low Density/No Shelter, and Low Density/Shelter (Fig. 1). Enrichment structures were constructed using submerged shredded black polyethylene material, covering approximately half the tank area and volume. The shreds were bundled for easy removal and cleaning, each bundle consisted of 100 shreds (50×7 cm) threaded on a 150 cm long polyester rope. The material was chosen for its chemically inert and easy handling and cleaning properties. The structures created a heterogeneous water flow with both vertical and horizontal cover, thus providing a 3D structure

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¹ For details of spatial placing of the treatment tanks, See Figure S1 in supplement
in which the fish could move freely. This shelter design was expected to minimize effects of fighting for access to shelter and was based on an earlier study (Näslund et al. 2013). To enable evaluation of long term effects (Ahlbeck Bergendahl, Salvanes & Braithwaite 2016) the fish were placed in the four different treatment as parr in autumn (Oct 8, 2012) and kept there the following 33 weeks, during which different behavioural and physiological traits were tested on subsamples of fish until final release as smolts into the natural habitat of the River Imsa May 24, 2013.

All tanks were supplied with flow through, naturally tempered water from a nearby lake. Commercial food pellets were given in excess from automatic feed dispensers (Ewos No. 505, Ewos AS, Skårer, Norway) and the light regime was adjusted to follow natural daylight rhythm. Animals were cared for in accordance with the “Guide for the Care and Use of Laboratory Animals” (1996), and the experiments conducted according to national regulation for treatment and welfare of experimental animals under license no. 051 granted by the Norwegian Animal Research Authority to the NINA Research Station, Ims.

Growth, fin damage and in-tank oxygen

Fork length ($L$; precision: 1 mm) and wet mass ($W$; precision: 0.1 g) were measured on all fish at the start of the experiment, showing no statistical difference between the groups. To be able to monitor individual growth and dorsal fin deterioration, 70 fish per tank were tagged with passive integrated transponders, PIT-tags (Biomark, Inc., Meridian, Idaho, USA). For quick identification of tagged and non-tagged fish, the adipose fin was removed on the remaining fish. Dorsal fin damage was used as an indication of internal tank aggression and scored from 1 to 3,

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2For further details see “Maintenance” in materials and methods section in supplement

3For further details of size and growth see Table S1 in supplement
with 1 = negligible damage, 2 = less than 50% of fin area eroded, and 3 = more than 50% of fin area eroded (cf. MacLean et al. 2000). Analyses were performed on the change in fin score i.e., fin deterioration over time where 0 = no change in fin damage score, 1 = increase in fin damage score, -1 = decrease in fin damage score. Analyses of growth and fin damage were performed from data collected from the PIT-tagged fish on Oct 10, 2012 and the final measurement set no later than March 1, 2013, to avoid stress and handling prior to release. To measure if animal density or the sheltering structures affected the water quality, in-tank oxygen levels (mg × l⁻¹) were measured May 13, 2013⁴. All tanks had high levels of water oxygen, ranging between 7.9-9.4 mg × l⁻¹.

### Blood sampling procedure

For each blood sampling occasion, the total number of fish sampled from each tank was netted simultaneously and immediately anesthetized in metomidate (6 mg × l⁻¹). After length and weight were registered, samples were taken from the caudal vein, using heparinized syringes. Extra care was taken not to disturb the tanks prior to sampling and all samples were taken within the window of cortisol excretion and during daytime (Gamperl et al. 1994). The plasma was separated by centrifugation and stored in –80 °C until analysis. Samples for basal cortisol levels were taken on four different occasions, Dec 11, 2012; Jan 22, 2013; Feb 25, 2013 (n = 18) and as pre-smolts 10 days before release, May 13, 2013 (n = 12).

### In-tank stress test

To measure the effects on plasma cortisol levels after applying an in-tank disturbance, an additional subsample was taken on Feb 28, 2013 (n = 18). The stressor was created through vibrations and a whirlpool within the water body, using a hand held electric screw driver with an

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⁴For further details see “In-tank oxygen” in materials and method section in supplement
attached, 40 cm long J-shaped metal rod, rotating at 200 rpm. For the enriched tanks, the rod was placed in the area without shelter and for the barren tanks in the corresponding place.

The disturbance was applied for 2 min for each tank and blood samples were taken 30 min post-stress.

**Plasma cortisol levels**

Plasma cortisol concentration was measured using a radioimmunoassay (Young 1986) modified by (Sundh et al. 2011). The lower detection limits of the RIAs ranged between 0.8 and 1.0 ng × ml\(^{-1}\) and samples below these concentrations were appointed their specific limit value.

**Intestinal barrier function**

To examine the intestinal physiology and barrier function of the fish before release into the wild, the *in vitro* Ussing chamber method was used (Sundell et al. 2003; Sundell and Sundh 2012). In short, the intestine was dissected out, cut open longitudinally and separated into its proximal and distal parts. Each intestinal segment was mounted between two half chambers representing the mucosal (luminal) and the serosal (blood) side.

The integrity of the intestine is assessed through transepithelial resistance (TER), a measurement of the paracellular permeability of charged molecules and as paracellular diffusion of the uncharged inert hydrophilic marker molecule, mannitol. Nutrient transport can be assessed as amino acid uptake from the mucosal to the serosal side. The hydrophilic \(^{14}\)C-mannitol (56.5 Ci × mmol\(^{-1}\), 3.7 MBq × ml\(^{-1}\)), and amino acid lysine (\(^{3}\)H-Lysine (91.6 Ci × mmol\(^{-1}\), 37 MBq × ml\(^{-1}\)), (NEN/Amersham) were added at \(t = 0\) where after transport rates and TER were recorded for 150 min.

\(^{5}\)For further details see “Plasma cortisol level” in materials and methods” section in supplement

\(^{6}\)For details see “Intestinal barrier function” in materials and methods section in supplement
Shelter seeking trials

To quantify shelter seeking behaviour, the same set-up and protocol was used as in Näslund et al. (2013) with a few alterations. The fish were tested individually and released on one side of a tank divided by a mesh with holes, through which the fish could swim. On the opposite side of the divider two shelter structures (opaque plastic tubes, length = 12 cm, diameter = 4 cm) were placed. 20 fish from each replicate tank \(n = 60\) were tested and divided systematically between the 16 test tanks. The position of each fish was manually observed and given a binomial score, “using shelter” or “not using shelter” every 10 min for 1 h. The score: “using shelter” was given if a fish was located at least within one body width distance from the shelter. If a fish was using the shelter at any of the observations it was scored as a “using shelter”. The trials were performed twice, once as parr, 26-27 of February (water temp 2°C) and then repeated in the pre-smolt stage 10-11 of May, using a different set of individuals (water temp 8°C).

Silvering index

To document silvering index (smolt status scored by visual markers), the left side of each fish was photographed using a digital camera with a built-in flash (Olympus Tough TG-1 iHS, Olympus Corp., Tokyo, Japan) during the last sampling May 13, 2013, 12 days before release \(n = 12\). Visual assessment was performed individually by three persons where “the principle of majority rules” was used when in disagreement. It was based on a four-grade scale from 1 (indicating fully visible parr marks and no silvering) to 4 (indicating full silvering and no visible parr marks) following Staurnes et al. (1993).

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7For details of tank design see Figure S2A in supplement

8For details see “Shelter seeking” in materials and methods section in supplement

9For details on scoring criteria see Figure S2B in supplement
**Smolt migration**

To measure downstream migration success, all the PIT-tagged fish \( n_{LDNS} = 193, n_{HDNS} = 192, n_{LDS} = 151, n_{HDS} = 189 \) were released into the River Imsa\(^{10}\) at a site 750 m above a permanent Wolf trap (inclination 1:10; apertures 10 mm). The trap is positioned 200 m upstream from the river outlet and captures all the fish exiting the river, the whole water volume of the river passes the trap and the fish cannot move upstream because of an unpassable waterfall.

The time of release (May 24, 2013) was decided using standard hatchery practices, i.e. based on fish swimming behaviour with the current in the tanks. The release date corresponded well with the wild smolt migration in the river this year (2013) that took place between the beginning of April and the end of May\(^{11}\). All fish were released at the same time (13.00- 13.15, water temp 11.3 °C, water velocity: 3.53 m\(^3\)/s)\(^{12}\) and the migration rate and success was monitored by catching the descending fish in the trap, which is emptied at least twice a day (08.00 and 15.00) all year round.

**Data treatment and statistical analysis**

**All data**

Assumptions regarding normality of residuals and homogenous variances were considered to be satisfactory based on inspection of Q-Q-plots, boxplot symmetry and spread. The threshold for significance was \( p = 0.05 \). When not stated otherwise, all statistical analyses were run in R version 3.0.2 (R Core Team 2013). For the LMM analysis the package ‘nlme’ (Pinheiro et al.

\(^{10}\)For descriptions of River Imsa see “Migration” in material and method section in supplement

\(^{11}\)For detailed information on wild smolt migration 2013, see Figure S3 in supplement

\(^{12}\)For detailed information on Imsa River water properties spring 2013 see Figure S4 in supplement
2013) was applied, while analysis based on GLMMs were performed by the package ‘lme4’ (Bates et al. 2013).

### Growth

Growth was analysed applying linear mixed effects models (LMMs) with *Final size* (body length and body mass in March) as a dependent variable, *Initial size* as a covariate, *Density* and *Shelter* as fixed factors, and *Tank* as a random factor $^{13}$. 

### Plasma cortisol data

This data was analysed using stepwise simplifications of LMMs or generalized least square (GLS) models$^{14,15}$. The beyond optimal statistical model included *Density* and *Shelter* and their interaction as fixed factors, body size (*Length*) as a covariate, and *Tank* as random factor. When interaction effects were significant, the two-way design was divided into the four combinations; High Density/No Shelter, High Density/Shelter, Low Density/No Shelter and Low Density/Shelter as treatment factors to perform post-hoc tests. Basal cortisol data was analysed separately for each sampling occasion (December, January and February).

### Intestinal barrier function

The intestinal barrier function data was analysed in the same manner as the plasma cortisol data but only GLS models were applied since tank effects were clearly insignificant ($p > 0.25$). For lysine uptake and anterior intestine mannitol uptake, variance components had to be added to account for heteroscedasticity (lysine: residual variance increasing with body size; mannitol: residual variance increasing with fitted value).

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$^{13}$For further details see “Growth” in data treatment and statistical analysis section in supplement.

$^{14}$For further details see “Plasma cortisol” in data treatment and statistical analysis section in supplement

$^{15}$For further details on statistical models see Table S2 in supplement
Shelter seeking

Shelter seeking behaviour was analysed using a binary logistic regression within the generalized linear mixed model (GLMM). The GLMM analyses started with a global model containing Shelter, Density, Month, and all their interactions, as well as Tank nested within the Density×Shelter and interaction added as a random effect block. To gain power, the global model was reduced by sequentially removing non-significant interaction terms\textsuperscript{16}. The analyses were performed using IBM SPSS Statistics 22 (SPSS, Inc., an IBM Company, Armonk, New York).

Fin deterioration, silvering index and migration

These data sets were analysed using a stepwise simplification of generalized linear mixed models (GLMMs) with a binomial probability distribution\textsuperscript{15,17}.

Results

Growth

As indicated by the overall size\textsuperscript{3}, adding shelter had a negative effect on growth (Length: $L_1 = 16.4, p < 0.001$, Mass: $L_1 = 5.6, p < 0.001$), (Fig. 2). In barren tanks, low density had a positive effect on growth (Density×Shelter interaction, Length: $L_1 = 5.80, p = 0.016$, Mass: $L_1=7.68, p = 0.006$), however no effect of density was found in the shelter tanks (post-hoc tests, Length: $L_1 = 0.17, p = 0.7$, Mass: $L_2 = 1.0, p = 0.6$)

\textsuperscript{16}For further details see “Shelter seeking” in data treatment and statistical analysis section in supplement

\textsuperscript{17}For further details see “Fin deterioration, silvering index and migration” in data treatment and statistical analyses section in supplement
In addition, there was a significant interaction effect of Initial mass and Shelter \((L_1 = 13.2, p < 0.001)\) on mass growth, with the larger individuals suffering a larger growth disadvantage by shelters compared to the smaller ones. For length growth this interaction was close to significant \((L_1 = 3.52, p = 0.06)\).

**Plasma cortisol**

In the in-tank stress test, the shelter group had significantly lower plasma cortisol concentrations compared to the no shelter group \((L_1 = 20.3, p < 0.0001)\), (Fig. 3). There was also a significant effect of body length \((L_1 = 9.0, p = 0.003)\), with higher levels for larger individuals \((\beta = 0.55 \pm 0.18 \text{ SE})\).

In the basal measurements a small but significant effect due to shelter was found in December \((L_1 = 25.6, p < 0.0001)\), where fish reared without shelter had slightly higher cortisol levels\(^{18}\). Despite large differences between the groups in January, no significant treatment effect was found when tank effects were included in the model. However, in two tanks from the Low Density/No Shelter group all individuals except one had levels elevated from what is generally considered basal \((\text{unstressed} < 10 \text{ ng} \times \text{mL}^{-1}; \text{Iwama 1998})\). In February, the larger individuals had significantly higher cortisol values \((L_1 = 11.4, p < 0.0001)\) and there was a tendency for slightly higher cortisol levels in the no shelter group \((L_1 = 3.2, p = 0.07)\).

**Shelter seeking behaviour**

Despite large differences in shelter seeking behaviour among treatments in February, indicating higher shelter frequency in the Low Density/Shelter treatment, no significant effect was found when tank effects were included in the model (Fig. 4). However, there was a difference between

\(^{18}\)See Figure S5 in supplement
months, where fish in Feb (parr) sought shelter to a higher degree compared to fish in May (pre-smolts) (F_{1,441} = 4.472, p = 0.035)^{19}.

**Intestinal barrier function**

The transepithelial resistance (TER) of the intestine was lower in the high density compared to the low density group, irrespective of intestinal region; proximal, (L\textsubscript{1} = 9.7, p = 0.002), (Fig. 5A), distal (L\textsubscript{1} = 15.0, p < 0.001), (Fig. 5B). No significant difference in permeability for mannitol was found\(^{20}\). For lysine up-take rate, there was an interaction effect in the proximal intestine (L\textsubscript{1} = 6.7, p = 0.01), (Fig. 5C) with the Low Density/No Shelter group showing a lower absorption rate than all other treatment groups (post-hoc tests: L\textsubscript{1} > 8.8, p < 0.001) and the High Density/No Shelter group having a higher absorption rate than the Low Density/Shelter group (post-hoc test: L\textsubscript{1} = 4.2, p = 0.04). In the distal intestine there was a main treatment effect with the high density group having a higher absorption rate compared to the low density group (L\textsubscript{1} = 10.9, p = 0.001), (Fig. 5D).

**Fin damage, smolt stage cortisol and silvering index**

For fin deterioration, there was an interaction effect (\chi^{2} = 9.84, p = 0.002) with the High Density/No Shelter group having higher deterioration than the other groups (post-hoc tests: \chi^{2} > 7.44, p < 0.006) which in turn did not differ from each other (post-hoc tests: \chi^{2} < 1.5, p > 0.22) (Fig. 6). There were no significant treatment effects on smolt stage cortisol\(^{21}\), or silvering

\(^{19}\)For further details see "Shelter seeking" in the Result section in supplement

\(^{20}\)See Figure S6 in supplement

\(^{21}\)See Figure S7 in supplement
Furthermore no relation to body size was found (plasma cortisol: $L_1 = 1.0$, $p = 0.3$, silversing index: $\chi^2 = 0.13$, $p = 0.7$).

Migration

The proportion of smolts successfully migrating (i.e. caught in the trap above the river mouth) was as follows: High density/No shelter 29% (53 out of 192), Low density/No shelter 32% (61 out of 193), High density/Shelter 15% (29 out of 189) and Low density/Shelter 24% (37 out of 151). Stepwise simplification of the full GLMM model with density, shelters and individual body length as a covariate, resulted in the only significant effect being body length ($\chi^2 = 13.96$, $p < 0.001$) and shelter ($\chi^2 = 5.63$, $p = 0.018$). Migration probability was higher for larger fish and for fish reared without shelter enrichment (Fig. 7). There were no significant three- or two-way interaction effects or significant effect of density ($\chi^2 = 2.07$, $p = 0.15$). There was a close to significant interaction effect of Density and Shelters ($\chi^2 = 3.41$, $p = 0.064$), indicating that the negative effect of shelters is mainly pronounced at high density (Fig. 7). The following year, 2014 (April-May) 15 fish were caught as 2 year old smolt. The group contained individuals from all groups: (4 fish from High Density/No Shelter; 4 from High Density/Shelter; 5 from Low Density/No shelter and 2 from Low Density/Shelter. This indicates that the majority of the fish that did not migrate in 2013 was probably killed by predation or did for some reason not seem to survive the following winter.

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$^{22}$See Figure S7 in supplement

$^{23}$For further details on migration pattern see Table S3 in supplement
Discussion

The present study shows that changes to the captive environment can affect both physiological and behavioural traits connected to welfare and post release performance of Atlantic salmon. Compared to conventional rearing, a lower animal density resulted in increased growth, decreased fin damage and improved intestinal barrier function, while in-tank shelter lowered stress hormone levels and fin damages. Thus, it seems likely that reduced density as well as shelter enrichment has the potential to produce a more robust phenotype. However, in-tank shelter had negative effects on growth rate, especially at high density. Furthermore, shelters, especially when combined with high density, also had a negative effect on migration success. This suggests that structural enrichment, in the form and time span used in this study should be avoided in combination with high densities of fish.

Basal cortisol

In January, an elevation of plasma cortisol above resting levels (Iwama 1998) was found in two out of three of the Low Density/No Shelter tanks; however, the overly large tank effects prevented detection of a statistical difference. The result is however in line with previous results from the same farming facility, where parr living at similar densities had higher resting cortisol levels in barren compared to shelter enriched tanks (Näslund et al. 2013). This suggests that keeping fish at low densities without shelter can result in sporadic stress, which might be induced by conspecific aggression (Øverli et al. 1999) or husbandry-related disturbances. The physiological relevance of the difference in basal cortisol levels found in December, between the shelter and no shelter treatment is unclear since the levels in all groups are below what is usually considered as “resting or basal levels” (Iwama 1998). In May, all groups show an
expected elevation connected to smolt development (Langhorne and Simpson 1986), with no difference between the treatments.

**In-tank stress test**

The cortisol response from the in-tank stress test clearly supports the hypothesis that shelter can protect against captivity-related disturbance. The stressor was designed to simulate potentially disturbing hatchery activity, with the aim to create equal vibrations and noise between the treatments, whereas the visual experience differed. The lower cortisol response in the shelter group is therefore probably caused by visual shielding and/or by the comfort of having access to shelter (Weiss 1968; Millidine, Armstrong and Metcalfe 2006; Kekäläinen et al. 2008). Within conservation programs there is often an incentive to reduce human contact, stress and domestication (Carter and Newbery 2004; Rodriguez et al. 1995) and it has been shown for a variety of species that opportunity for concealment in captivity is important for optimal well-being (Morgan and Tromborg 2007). Accordingly, this study shows that shelter is an important factor in reducing stress caused by human activity, also for fish and that providing access to shelter should be considered when designing rearing environments.

**Fin damage**

Over winter (Oct-Mar) the High Density/No shelter group had increased dorsal fin damage, whereas all other groups improved their fin status. This indicates a higher aggression level for this conventionally reared group (Tumbull et al. 1998). In tanks that contain structure and shelter, the visual field and interference from conspecifics is reduced (Imre et al. 2002; Morgan and Tromborg 2007) and it is probable that shelter can both prevent and break up an ongoing attack if the target has the opportunity to escape and hide. Reduced density, on the other hand, may increase familiarity between individuals (Brockmark and Johnsson 2010), which in turn
may facilitate stable social structures and thereby also reduce aggressive acts (Johnsson 1997; Griffiths et al. 2004). Both the stress inflicted by high aggression (Morgan and Tromborg 2007) and the subsequent breaches in the skin barrier can potentially result in a higher susceptibility to disease when in the captive environment (Schneider and Nicholson 1980) as well as after release (Fridell et al. 2007) for the conventionally reared High Density/No Shelter group.

**Intestinal barrier function**

When the intestinal barrier function was tested just prior to release as smolts in May, individuals raised at high density had considerably lower transepithelial resistance compared to the low density groups. Even though no sign of chronic elevation of plasma cortisol was found, a lower intestinal resistance can be a sign of prolonged stress and impaired welfare (Sundh et al. 2010; Segner et al. 2012). During long term, low-intensive stress, habituation of the corticosteroid system can occur through negative feed-back mechanisms on the hypothalamic-pituitary-interrenal axis. This would generate a decrease in plasma cortisol over time even though the stressor is still present (Segner et al. 2012; Dickens and Romero 2013). At high densities, general aggression is often high (MacLean et al. 2000; Johnsson et al. 2014), supported here by the higher fin damage in the High Density/No Shelter group, which could result in a chronic stress situation. High rearing densities and social stress have also been shown to negatively affect the intestinal barrier, both for Atlantic salmon (Sundh et al. 2009) and other teleost fishes (Peters 1982). In addition to revealing reduced welfare, an impaired intestinal barrier may compromise disease resistance, working as an infection route for pathogens (Berg 1995; Velin et al. 2004). Indeed, for Atlantic salmon, mild chronic stress in the freshwater stage has been shown to increase disease susceptibility and mortality in the forthcoming seawater phase (Fridell et al. 2007).
A higher stocking density could also lead to a lower water quality which in turn could affect the intestinal barrier negatively (Niklasson et al. 2011); however no sign of differences among tanks was seen in water oxygen concentration. Since no difference was found when comparing shelter and no shelter treatments independent of density, shelter structures as such did not seem to affect the threshold for negative effects of high density.

**Growth and nutritional up-take**

In contrast to some earlier studies (Brockmark et al. 2007; Salvanes et al. 2013) but in line with others (Fast et al. 2008), shelter in this experiment affected growth negatively. Although the enrichment design was successful in creating shelter both from conspecifics and human disturbance, it might still not be ideal for the growth and development of juvenile Atlantic salmon (Kalleberg 1958). For salmonids, growth is generally considered an adequate fitness-correlate as it affects other life history traits such as survival (Friedland et al. 2009) and fecundity (Jonsson et al. 1996). In the wild, the trade-off between feeding to maximize growth and sheltering to maximize survival is well known (Teichert et al. 2010). It is possible that growth in this study was depressed by risk sensitive behaviour (Kemp et al. 2005). The fact that the fish, even in the absence of predators, seem to favour hiding instead of eating and growing, suggests a high innate motivation to express sheltering behaviour (Griffiths and Armstrong 2002).

In line with earlier studies, sheltering structures limited the growth of larger individuals more than smaller (Brockmark et al. 2007). Enrichment structures restrict visibility, which can make it more difficult for dominant and larger individuals to monopolize food (Jobling 1985), it may also lower the advantage of being aggressive (Höjesjö et al. 2004), perhaps promoting...
phenotypes with a wider spectrum of behavioural strategies (McDougall et al. 2006). In the no
shelter environment, high density had a negative effect on growth. Growth rate is often
negatively correlated with animal density and might be caused by depressed food-intake caused
by intraspecific competition, (Fenderson and Carpenter 1971; Brockmark and Johnsson 2010)
and/or a possible lower food conversion efficiency caused by stress (Ellis et al. 2002; Leal et al.
2011). In support of the latter, the group with the highest growth rate (the Low Density/No
Shelter) also had the lowest nutrient uptake rate in the proximal intestine.

In the distal intestine, there was a general effect of density with a higher uptake rate of lysine in
the high density group. The kinetics of amino acid absorption differs between intestinal regions,
with the proximal intestine being the major organ for active nutritional absorption (Loretz 1995).
The higher uptake rate in the distal intestine of the high density group thus merely suggests an
increased passive paracellular permeability, which is well in line with the TER data and further
supports a decreased intestinal integrity in the high density group.

**Shelter seeking behaviour**

In February, the Low Density/Shelter group, showed a tendency towards a higher shelter seeking
behaviour. This is in line with a previous study on parr raised at corresponding density (Näslund
et al. 2013) and thus suggests a biological significance even if not statistically secured. Some
beneficial behavioural effects from adding shelter may only be expressed at reduced rearing
densities. Previous studies have shown that a lower rearing density can benefit cognitive traits
such as feeding on novel prey and predator avoidance through sheltering (Brockmark et al. 2010)
as well as increased post release survival (Brockmark et al. 2010; Brockmark and Johnsson
2010).
Fish in May, on the other hand, were less inclined to shelter regardless of rearing environment. This may be a result of a general increase in activity as the fish are changing from bottom living parr into free-swimming smolts (Thorstad et al. 2012). The fish were also observed to utilize the sheltering structures within the tanks to a lower degree during May (personal observations). Adjusting the captive environment to different life-stage specific requirements, e.g. provide shelter only during the fry and parr stage, when also cleaning is less frequently needed, might serve as a more efficient hatchery practice. For smolts, other types of enrichment, such as variations in water current strength, could instead be more beneficial (Hyvärinen and Rodewald 2013).

**Migration**

Migration behaviour was strongly correlated to the size of the fish, with larger fish showing superior migration success across all treatments. This size dependency is in accordance with earlier studies on the same age class (1+ smolts), where it has been argued that smaller fish might not be fully smoltified or more sensitive to predation (Hansen and Jonsson 1985; Kallio-Nyberg et al. 2004). In the present study, no correlations between size and the smolt status indicators, plasma cortisol and body silvering were found, suggesting that predation or behaviour might be more plausible factors restricting the migration. In addition to the general size effect, the shelter groups had a significantly lower migration success. This effect was however mainly driven by the High Density/Shelter group, where lower migration was displayed by fish of all sizes and can therefore not be attributed to any size differences. One possible explanation might be a higher frequency of sheltering behaviour once released into the natural stream for this group. Negative effects of sheltering structures on survival during migration have been shown for Chinook salmon (*Oncorhynchus tshawytscha*)
where increased mortality was suggested to stem from usage of in-stream shelters already occupied by predators (Berejikian et al. 1999). In the present study however, all groups showed equally low motivation to seek shelter in the controlled shelter seeking trials in May and also displayed a low motivation to shelter in their rearing tank (personal observations). Previous studies on interaction effects between animal density and enrichment structures in fish are limited (Näslund and Johnsson 2016), but show similar results as the present study with no or negative effects when combining structural enrichment and high animal density (Brockmark et al. 2007; Brockmark et al. 2010; Hoelzer 1987). For example, brown trout (Salmo trutta) reared with in-tank structure at high densities were half as likely to seek shelter after a simulated predator attack and half as likely to survive in a natural stream, compared to the low density shelter group (Brockmark et al. 2010). Similarly, Atlantic salmon, at high density with shelter, grew less, had more fin damage and lower survival in sea water, compared to salmon at low density and shelter (Brockmark et al. 2007). Other studies showing positive effects of in-tank structure on salmonid performance, do indeed apply lower animal density than standard practice (Näslund et al. 2013; Ahlbeck Bergendahl, Salvanes & Braithwaite 2016; Karvonen et al. 2016).

Positive effects of structural enrichment on Atlantic salmon migration have also been reported (Hyvärinen and Rodewald 2013). This study however, did not assess interaction between density and structures, the fish were larger 2+ smolts and also combined sheltering structures with other types of enrichment, such as changes in water velocity. In addition, this study used very low densities during the final part of the study.

It is possible that the inferior migration success seen in the High Density/Shelter group was caused by prolonged crowding, causing stress that can result in maladaptive post-release behaviour (Teixeira et al. 2007; Gaikwad et al. 2011). This has been shown in rearing
environment similar to the present (Brockmark et al. 2007). Although the sheltering structures in the present study were designed to provide access for all fish, individual space declines with increasing density. A presence of long-term stress in the High Density/Shelter group was also supported by the transepithelial resistance data, as discussed above.

In nature, increased habitat complexity has been linked to higher population density for Atlantic salmon (Teichert et al. 2010). Therefore it seems intuitive that this should allow for an increased stocking density also in captivity as seen in other species (Teng and Chua 1979). However, this does not seem to apply for the unnaturally high densities used in conventional salmon hatcheries and through a structure-induced increase in density one might be at risk of further enhancing negative high density effects. The inferior post-release performance of the High Density/Shelter group highlights the importance of carefully examining modifications to the captive environment; even though they may seem intuitive or “nature-like”.

In conclusion, a lowered rearing density, both with and without shelter, show promising results, with significant or strong trends towards positive effects on intestinal barrier function, sheltering behaviour, stress hormone levels and intra-specific aggression, all which may help to produce more resilient and robust salmon for release.

Nonetheless, shelter had negative effects on growth, and especially at high densities, in-tank shelters had negative effects on post release performance measured as smolt migration. Thus it seems that combining this type of structural enrichment with high rearing densities should be avoided for Atlantic salmon and that structural enrichment will not circumvent negative effects of high stocking density. The intestinal barrier function data and the higher prevalence of fin damage in the conventionally reared group (High Density/No Shelter) suggest that an impaired
disease resistance might be one potential factor causing the generally low sea survival of released
fish from hatcheries. This study further supports the call for investigating both behaviourally and physiologically
relevant outcomes of conservational management decisions (Blumstein and Fernández-Juricic
2004; Metcalfe et al. 2012), calling for future studies examining the effects of stress and disease
resistance also after release into the wild.
To enhance the welfare and quality of salmonids released for conservation purposes, we
recommend that conventional rearing densities should be reduced and that more research is
needed regarding both design and timing of in-tank shelter applications.

Acknowledgements
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References


http://cran.r-project.org/web/packages/lme4/index.html.


Figure legends

**Fig. 1.** Photographs showing treatment tanks of Low Density/No shelter and Low Density/Shelter together with a schematic picture of the whole experimental set-up. High density = 150 ind/m², Low density = 50 ind/ m².

**Fig. 2.** Individual length (A) and mass (B) growth from Oct-March. Shelter had a negative effect on growth and low density had a positive effect on growth in no shelter tanks. HD = High Density, LD = Low Density, NS = No Shelter, S = Shelter, (n = 210).

**Fig. 3.** Circulating plasma cortisol levels following human induced in-tank disturbance (stress) compared to basal levels (basal), (n = 18). Values show averages with 95% confidence intervals. Different letters indicate significant differences (p < 0.05).

**Fig. 4.** Proportion of fish using shelter in a novel environment both as parr (Feb) and pre-smolts (May), (n = 60). The fish were placed in a shelter seeking arena divided in two sections by a mesh with holes. The fish and the sheltering structures were placed on opposing sides and shelter seeking frequency was observed over 1 h. Asterisk (*) indicates significant difference (p < 0.05).

**Fig. 5.** Intestinal barrier function measured through trans-epithelial resistance, TER (A, B) and intestinal nutritional up-take rate of the amino acid $^3$H-Lysine (C, D) as pre-smolts in May, (n = 12). Bars show averages with error bars denoting 95% confidence intervals. Asterisk (*) and different letters indicate significant differences (p < 0.05).
Fig. 6. Conspecific aggression measured through change in dorsal fin score between October and March. Positive values demonstrate an increase in fin damage and negative values demonstrates an improved fin status, \((n = 210)\). Box hinges represent the first and third quartiles and the band within the box, the second quartile (median). Whiskers represent the data within, while dots represent data points 1.5 interquartile range away from the box hinges. Different letters indicate significant differences \((p < 0.05)\).

Fig. 7. Probability of migration success as smolts in the River Imsa in May, plotted against body length in March. Migration probability was significantly lower for smaller fish and for fish reared with in-tank shelter, especially at high density. \(n_{\text{LDNS}} = 193, n_{\text{HDNS}} = 192, n_{\text{LDS}} = 151, n_{\text{HDS}} = 189\). HD = High Density, LD = Low Density, NS = No Shelter, S = Shelter.
Figure 1.
Figure 2.

A

B

Body length (mm) March

Body mass (g) March

Initial body length (mm) Oct

Initial body mass (g) Oct

LDNS

HDNS

LDS

HDS
Figure 3.

The graph shows the cortisol levels (ng/ml) in different conditions. The x-axis represents the different density levels: High Density and Low Density. The y-axis represents the cortisol levels ranging from 0 to 60 ng/ml.

- **Basal** and **Stress** conditions are compared under High Density and Low Density.
- **No Shelter** and **Shelter** conditions are indicated with different markers.
- The graph includes error bars indicating variability in the data.

Letters **a**, **b**, and **c** are used to denote significant differences between the groups.
Figure 4. Proportion of shelters used by fish in February (Feb) and May, with high and low density conditions. The figure shows a significant difference in shelter use between high density and low density conditions in May, indicated by * p<0.05.
Figure 5.
Figure 6.

Box plots showing fin deterioration (Oct-March) for High Density and Low Density with and without shelter. Letters indicate significant differences among groups.
Figure 7.

![Graph showing predicted probability vs. body length for different categories (LDNS, HDNS, LDS, HDS).]
Electronic supplement to: Born to be wild: effects of rearing density and environmental enrichment on stress, welfare and smolt migration in hatchery reared Atlantic salmon

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Material and methods

1 Fig. S1. Schematic picture showing treatment and tank placement within the hatchery facility. HD = High density, LD = Low density, NS = No shelter, S = Shelter.

2 Maintenance
The sheltering structures were placed on the opposite side of the tank to the food dispenser and water inflow. All tanks were subjected to daily cleaning (except on sampling days) which included water level reduction (down to 8-10 cm water depth) and scrubbing of the tank. In addition, enrichment structures were lifted out of the tanks and quickly cleaned with a water hose when considered necessary (up to twice a week during the growth season and every second week during the coldest winter temperatures). During the first week, the tank cleaning procedure was not optimized and some fish dropped on the floor when the plastic tare was lifted out of the tanks. This problem was sorted out by lifting the artificial kelp into a plastic box. However, a few individual fish were returned to the wrong tanks. Hence, at the last size measurement, 7 individuals were found in a different tank than
where they were originally placed. In addition, some individuals (0-3) in each tank had either died or lost their tag. In one of the tanks (High Density/No Shelter), 8 individuals died during the experiment. Misplaced individuals and individuals that had lost their tag were excluded from the statistical analysis on growth and migration behaviour. One of the Low Density/Shelter tanks suffered mortality of 56 fish on May 4, 2014, due to low water level caused by failing to return the stand pipe plug to the outlet flow of water, after cleaning the tank. The remaining fish in the tank seemed to recover quickly from this added stressor and there were no signs of the fish from this tank deviating in the following pre- and post-release performance tests and analyses. This however, ultimately led to a somewhat smaller sample size being released for the migration study from the Low Density/Shelter group.

Table S1. Growth data
Length and weight data from PIT-tagged fish in October 2012 and March 2013. Mean values with ±95%CI = 95% confidence intervals, Length = fork length, Mass = achieved wet mass. HD = High Density, LD = Low Density, NS = No Shelter, S = Shelter.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length (mm)</th>
<th>Weight (g)</th>
<th>Condition factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct 8-2012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDNS</td>
<td>113.48</td>
<td>18.89</td>
<td>1.27</td>
</tr>
<tr>
<td>HDS</td>
<td>113.73</td>
<td>18.96</td>
<td>1.27</td>
</tr>
<tr>
<td>LDNS</td>
<td>113.46</td>
<td>18.95</td>
<td>1.27</td>
</tr>
<tr>
<td>LDS</td>
<td>114.04</td>
<td>19.26</td>
<td>1.27</td>
</tr>
</tbody>
</table>

| March 1-2013 |            |            |                 |
| HDNS        | 124.01      | 21.11      | 1.09            |
| HDS         | 121.72      | 19.16      | 1.05            |
| LDNS        | 126.83      | 23.10      | 1.12            |
| LDS         | 121.98      | 19.18      | 1.04            |

In-tank oxygen
In-oxygen was measured using a multi-parameter water quality meter (HI-9828; Hanna Instruments, Smithfield, Rhode Island, USA). The measurement was taken inside the sheltering structure and in the barren tanks in the corresponding place.

Plasma cortisol levels
Sheep anti-cortisol antibodies (Code: S020; Lot: 1014–180182; Guildhay Ltd., Guildford, Surrey, UK). Tritiated hydrocortisone-[1,2,6,7-3H(N)] (NET 396, NEN Life Sciences Products, Inc., Boston, Massachusetts, USA) was used as tracer and radioactivity measured in a β–counter (Wallac 1409 Liquid Scintillation Counter, Turku, Finland). Non-radioactive labelled cortisol standards were prepared from hydrocortisone (Sigma-Aldrich, St. Louis, Missouri, 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).
**Intestinal barrier function**
The method gives information regarding the electrophysiological properties as well as the diffusion and transport rate of substances across the epithelium. To ensure viability of the tissue, oxygenated ringer solution (Jutfelt et al. 2007) was added to each half-chamber in the Ussing chamber set-up and kept at the fish acclimation temperature, 8° C, using water filled cooling mantles. The proximal and distal parts of the intestine have different diameters, therefore chambers with different exposure area and volumes were used (proximal: 0.08 cm², 2 ml, distal: 0.75 cm², 4 ml). After mounting, the preparations were allowed a stabilizing period of 60 min, after which the Ringer solution was renewed (with added radioactive labelled markers). Measurements of TER were taken every 5 min together with transepithelial potential and short circuit current (to validate viability of the intestinal epithelia) and continued for 90 min. The ¹⁴C-mannitol and ³H-Lysine were added in the following volumes on the mucosal side; anterior intestine: 17.6 µl ¹⁴C-mannitol × ml⁻¹, 3.5 µl ³H-Lysine × ml⁻¹ resulting in a specific activity of 261.2 MBq × mmol⁻¹, posterior intestine: 4.7 µl ¹⁴C-mannitol × ml⁻¹, 1.0 µl ³H-Lysine × ml⁻¹ resulting in a specific activity of 67.2 MBq × mmol⁻¹.

To assess the transfer rate of the radiolabelled compounds across the epithelia, serosal samples (50 µl) were taken at t = 0, 20, 25, 30, 55, 80, 85 and 90 min (the removed fluid volume was replaced with fresh Ringer solution). The samples were put into scintillation vials and 4.5 ml of liquid scintillation fluid (ULTIMA GOLD™, PerkinElmer,Inc) was added and the radioactivity was assessed in a β–counter (Wallac 1409 Liquid Scintillation Counter, Turku, Finland).

The transfer rate of mannitol was measured as: apparent permeability ($P_{app}$) which calculates the diffusion rate (cm × s⁻¹) of ¹⁴C-mannitol using the equation:

$$P_{app} = \frac{(dQ/dT) \times (A \times C_0)^{-1}}{\text{equation 1}}$$

where $dQ/dT$ is the accumulation rate of ¹⁴C-mannitol on the serosal side
$dQ$ is measured as serosal concentration of ¹⁴C-mannitol
$C_0$ is the mucosal concentration of ¹⁴C-mannitol at t = 0
$A$ is the area of the intestinal segment.

The transfer rate of Lysine was measured as $T_{Lys}$ which calculates the transport rate (nmol · (min · cm⁻²)) of ³H-Lysine using the equation:

$$T_{Lys} = \frac{(dQ/dT) \times A^{-1}}{\text{equation 2}}$$

where $dQ/dT$ is the accumulation rate of Lysine on the serosal side
$dQ$ is calculated as serosal DPM of ³H-Lysine divided by the mucosal specific activity of ³H-Lysine (DPM × mmol⁻¹ Lysine)
$A$ is the area of the intestinal segment.
Shelter seeking
Repeated nettings of fish from tanks with and without shelter creates potential differences in handling stress. Therefore, to standardize the starting point of the shelter seeking trials all fish used were removed from their original tanks and transferred to barren tanks the night before the trials started. The trials were run over two days. For the May trials the openings were enlarged (3.1 cm × 3.8 cm) to allow the larger pre-smolts to pass through and seek shelter.

Migration
The 1 km long river Imsa supports a small wild population of anadromous Atlantic salmon that naturally migrates downstream into the Høgsfjord estuary. The river system has been used for studying the migration behavior of hatchery reared and wild Atlantic salmon for a long time (Jonsson & Jonsson 2014), (NINA 2014)
**Fig. S3.** Data showing the timing and number of wild migrating smolt of Atlantic salmon caught in the trap during spring 2013.

**Fig. S4.** Water temperature (°C) and water flow (m³ × s⁻¹) in the river Imsa during spring 2013.
Data treatment and statistical analysis

Growth
To account for decreasing variance with increasing initial size (amount of growth varied more for smaller individuals, Fig. 2), a variance component, $\text{varPower(form = ~Initial size)}$, was added to the model. This removed heterogeneity without transformation of variables. Starting with a full model including all interactions, insignificant terms were removed by a stepwise procedure, following Zuur et al. (2009). Significance of interactions and main factors were tested by likelihood ratio tests with a significance level of 0.05. Controlling for tank effects and initial size through model simplification of LMMs resulted in optimum models with $\text{Density, Shelter}$ and their interaction as significant or close to significant factors.

Plasma cortisol
When tank effects were clearly insignificant, defined by $p$-values larger than 0.25, $\text{Tank}$ was removed as random factor and tests were based on GLS models. Due to limited number of samples (48 fish), treatment interactions with body size and three-way interactions were not included to avoid overfitting. To adjust for heteroscedasticity, appropriate variance structures were added to the models if necessary, (see Table S2). Smolt stage and basal plasma cortisol were, $\log_e$ transformed to achieve normality of residuals, while no transformation was needed for the stress test cortisol values. When interaction effects were significant, the two-way design was divided into the four combinations; High Density/No Shelter, High Density/Shelter, Low Density/No Shelter and Low Density/Shelter as treatment factors to perform post-hoc tests. Pairwise comparisons were performed by pooling treatment groups one by one and testing if the simplification (pooling of groups) significantly reduced model performance, applying likelihood ratio tests with a significance level of 0.05.
Table S2, Statistical models
Details of statistical models applied for testing of significant effects of factors and covariates on the dependent variables: $\text{LogPC} = \log_e$ transformed plasma cortisol values, $\text{FinSC}= \text{change in dorsal fin score}, \text{Length} = \text{fork length in March}, \text{Mass} = \text{achieved wet mass in March}$. Variance components were added in both LMM and GLS models when needed to control for variance heterogeneity.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Stat. model</th>
<th>Fixed Effect Terms in Beyond Optimal Model</th>
<th>Random factors</th>
<th>Link-function</th>
<th>Variance component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>LMM</td>
<td>Initial length * Density * Shelter</td>
<td>1</td>
<td>Tank</td>
<td>-</td>
</tr>
<tr>
<td>Mass</td>
<td>LMM</td>
<td>Initial mass * Density * Shelter</td>
<td>1</td>
<td>Tank</td>
<td>-</td>
</tr>
<tr>
<td>FinSC</td>
<td>GLMM</td>
<td>Initial length * Density * Shelter</td>
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<td>Tank</td>
<td>Logit</td>
</tr>
<tr>
<td>Silvering</td>
<td>GLMM</td>
<td>Length * Density * Shelter</td>
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<td>Tank</td>
<td>Logit</td>
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<tr>
<td>Migration</td>
<td>GLMM</td>
<td>Length * Density * Shelter</td>
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<td>Tank</td>
<td>Logit</td>
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<tr>
<td>$\text{LogPC}_{\text{smolt}}$</td>
<td>GLS</td>
<td>Length + Density * Shelter</td>
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<td>-</td>
<td>none</td>
</tr>
<tr>
<td>$\text{LogPC}_{\text{Dec}}$</td>
<td>GLS</td>
<td>Length + Density * Shelter</td>
<td>ns</td>
<td>-</td>
<td>varIdent</td>
</tr>
<tr>
<td>$\text{LogPC}_{\text{Jan}}$</td>
<td>GLS</td>
<td>Length + Density * Shelter</td>
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<td>-</td>
<td>none</td>
</tr>
<tr>
<td>$\text{LogPC}_{\text{Feb}}$</td>
<td>LMM</td>
<td>Length + Density * Shelter</td>
<td>1</td>
<td>Tank</td>
<td>-</td>
</tr>
<tr>
<td>StressPC</td>
<td>GLS</td>
<td>Length + Density * Shelter</td>
<td>ns</td>
<td>-</td>
<td>none</td>
</tr>
</tbody>
</table>

Shelter seeking
To gain power, the global model was reduced by sequentially removing non-significant interaction terms, starting with the three-way interaction and then removing two-way interactions, starting with the one with highest $p$-value. None of the interaction terms were retained in the final model (all had $p > 0.15$). In addition a similar analysis was used, but without $\text{Tank}$ as a factor (generalized linear model; GLM). For the GLM, the same model reduction procedure was carried out but the final model contained only the $\text{Density} \times \text{Shelter}$ interaction. Significant interaction effects were evaluated using Holm-Bonferroni corrected pairwise contrasts.

Fin deterioration, silverying index and migration
The silverying index was given binomial values by recoding score 4 into 1 and scores 1-3 into 0. The starting model included $\text{Fork length}$ (in May), $\text{Density}$ and $\text{Shelter}$. For the migration data both two- and three-way interactions were included as fixed effects in the beyond optimal model, while only the $\text{Density} \times \text{Shelter}$ interaction was included in the initial models for fin deterioration and silverying index, due to the limited number of samples. To avoid pseudo-replication, $\text{Tank}$ was included as random factor in all analysis. Simplification of the initial model was performed by step by step removing insignificant terms following $\text{Zuur et al.}$ (2009).
Results

Fig. S5. Boxplots showing basal plasma cortisol concentrations for all treatments and tanks (Dec-Feb). **HD** = High Density, **LD** = Low Density, **NS** = No Shelter, **S** = Shelter, \((n = 18)\). Box hinges represent the first and third quartiles and the band within the box the second quartile (median). Whiskers represent the data within, while dots represent data points 1.5 interquartile range away from the box hinges. Asterisk(*) indicates significant difference \((p < 0.05)\).


c\(\text{\dag}\) Shelter seeking

When disregarding tank effects, there was an effect of Shelter, \((\text{Wald } \chi^2 = 4.058, p = 0.044)\) and a significant interaction \((\text{Wald } \chi^2 = 4.058, p = 0.044)\), indicating that fish reared in Low Density/Shelter had higher probability of seeking shelter (post-hoc test: \(p = 0.022\)). There was also an effect of Month \((\text{Wald } \chi^2 = 4.293, p = 0.038)\). The combined approach of the GLMM and the GLM suggest that there are strong tank effects, where some low density tanks with shelter perform particularly well in the shelter seeking trials, while others do not. Such a pattern can also be seen in the raw data.
Fig. S6. Intestinal barrier function measured through permeability of the paracellular marker molecule, \( ^{14} \text{C}-\text{mannitol} \) in the proximal and distal part of the intestine as pre-smolts in May, \((n = 12)\). Bars show averages with error bars denoting 95% confidence intervals.

Fig. S7. Basal plasma cortisol levels as pre-smolts in May \((n = 12)\). Box hinges represent the first and third quartiles and the band within the box, the second quartile (median). Whiskers represent the data within, while dots represent data points 1.5 interquartile range away from the box hinges.
**Fig. S8.** Silvering index (smolt status scored by visual markers) as pre-smolts in May (n = 12). Scoring was based on a four-grade scale, Smolt stage 1 = parr colouring (no fish scored as stage 1) up to Smolt stage 4 = full silvering.

**HD =** High Density, **LD =** Low Density, **NS =** No Shelter, **S =** Shelter.

**Table S3, Migration data**
Data showing number of fish caught in the smolt trap (successfully migrating) from the release date May 24th (Day 0+X) during 2013 divided by treatment and tank.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tank</th>
<th>Day 0</th>
<th>Day +1</th>
<th>Day +2</th>
<th>Day +3</th>
<th>Day +5</th>
<th>Day +6</th>
<th>Day +28</th>
<th># migrating</th>
</tr>
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<tbody>
<tr>
<td>HDNS</td>
<td>1</td>
<td>4</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>17</td>
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<td>10</td>
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<tr>
<td></td>
<td>5</td>
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References


