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ARTICLE

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 with wild conspecifics. To assess potential hatchery rearing improvements, behavioral and physiological effects of reducing animal density and adding in-tank shelter were investigated. Atlantic salmon (*Salmo salar*) parr were placed in barren or shelter-enriched tanks at high or low density up until release as smolts. Lowered density rendered positive effects on growth and intestinal barrier function, and both lowered 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.

Résumé : Les salmonidés élevés en écloserie relâchés en milieu naturel présentent généralement une faible capacité de survie par rapport à leurs conspécifiques sauvages. Afin d'évaluer des améliorations possibles à l'élevage en écloserie, les effets comportementaux et physiologiques de la réduction de la densité d'animaux et de l'ajout d'abris dans les bassins ont été étudiés. Des tacons de saumon atlantique (*Salmo salar*) ont été placés dans des bassins avec et sans abris, à des densités élevées ou faibles jusqu'à leur lâcher au stade de saumoneau. Une densité réduite produisait des effets positifs sur la croissance et la fonction de barrière intestinale, et la combinaison d'une densité réduite et de la présence d'abris réduisait le nombre d'agressions par des conspécifiques indiquées par les dommages aux nageoires. En outre, si la présence d'abris a entraîné une diminution des concentrations d'hormones de stress après une perturbation humaine, elle a également mené à des réductions de la croissance et du succès de migration des saumoneaux, un effet particulièrement marqué à de fortes densités. Nous suggérons donc que ce type d'enrichissement structural devrait être évité pour les saumoneaux de saumon atlantique gardés dans des conditions de forte densité et concluons qu'une réduction de la densité des animaux avec ou sans abris présente le meilleur potentiel de production de saumoneaux plus résilients pour l'empoissonnement. [Traduit par la Rédaction]

Introduction

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 postrelease performance are limited (Fischer and Lindenmayer 2000; Seddon et al. 2007). Atlantic salmon (*Salmo salar*) 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 with 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 with 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).

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Previous studies on lowered density and structural enrichment have shown positive effects through, for example, decreased aggression (Brockmark et al. 2007; Näslund et al. 2013). Furthermore, reduced density has been reported to result in improved antipredator behaviour (Brockmark et al. 2010) and increased survival after release (Brockmark et al. 2010; Brockmark and Johnsson 2010), whereas in-tank shelter has 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 shows no or even negative effects on, for example, postrelease performance (Berejikian et al. 1999; Brockmark et al. 2010; Näslund and Johnsson 2016), 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 postrelease success. The aim of this study was therefore to (*i*) investigate whether reduced animal density and structural enrichment affect growth, stress hormone responses, shelter-seeking behaviour, and intestinal primary barrier functions and to (*ii*) examine if these measures result 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 postrelease performance.

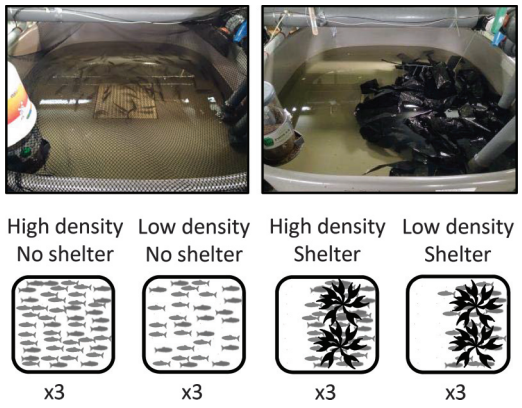
Materials and methods

Experimental fish and 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 8 October 2012, a total of 2400 fish were randomly divided among the four treatments, each with three 2 m^2 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 individuals·m^{-2}, mass density in May 2013: 14.4 kg·m^{-3}) and low density (50 individuals·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; for details of spatial placing of the treatment tanks, see Fig. S1 in the online supplementary data[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 cm × 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 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 et al. 2016), the fish were placed in the four different treatment as parr in autumn (8 October 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 on 24 May 2013.

Fig. 1. Photographs showing treatment tanks of Low Density–No shelter and Low Density–Shelter together with a schematic picture of the whole experimental setup. High density = 150 individuals·m^{-2}, Low density = 50 individuals·m^{-2}.



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. (For further details, refer to "Maintenance" in Material and methods section in supplementary data[1].)

Animals were cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" (Committee for the update of the Guide for the Care and Use of Laboratory Animals 1996), and the experiments were 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 among the groups. (For further details of size and growth, see Table S1[1].) To be able to monitor individual growth and dorsal fin deterioration, 70 fish per tank were tagged with passive integrated transponders (i.e., PIT-tags; Biomark, Inc., Meridian, Idaho, USA). For quick identification of tagged and nontagged 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, 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 10 October 2012 and the final measurement set no later than 1 March 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 13 May 2013. (For further details, see "In-tank oxygen" in Material and method section in online supplementary data[1].) All tanks had high levels of water oxygen, ranging between 7.9 and 9.4 mg·L^{-1}.

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2015-0515.

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 mass 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: 11 December 2012; 22 January 2013; 25 February 2013 (n = 18), and as presmolts 10 days before release, 13 May 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 28 February 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 attached 40 cm long J-shaped metal rod, rotating at 200 r·min^{-1} (1 r = 2πrad). 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 poststress.

Plasma cortisol levels

Plasma cortisol concentration was measured using a radioimmunoassay (Young 1986) modified by (Sundh et al. 2011). (For further details, see "Plasma cortisol level" in Material and methods section in online supplementary data[1].) The lower detection limits of the radioimmunoassays 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). This was performed on presmolts 10 days prior to release, 13 May 2013. 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 was assessed through transepithelial resistance (TER), a measurement of the paracellular permeability of charged molecules, and as apparent permeability (P_{app}), a measurement of the 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. (For details, see "Intestinal barrier function" in Material and methods section in online supplementary data[1].)

Shelter-seeking trials

To quantify shelter-seeking behaviour, the same setup 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. (For details of tank design, see Fig. S2A[1].) On the opposite side of the divider, two shelter structures (opaque plastic tubes, length = 12 cm, diameter = 4 cm) were placed. In total, 240 fish (20 from each replicate tanks, n = 60) were tested individually and systemically divided among 16 parallel test tanks. (For details, see "Shelter seeking" in Material and methods section in online supplementary data[1].) 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 from the shelter. (For details on scoring criteria, see Fig. S2B[1].) If a fish was using the shelter at any of the observations, it was scored as "using shelter". The trials were performed twice, once as parr, 26–27 February (water temperature = 2 °C) and then repeated in the presmolt stage on 10–11 May, using a different set of individuals (water temperature = 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 on 13 May 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).

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 at a site 750 m above a permanent Wolf trap (inclination 1:10; apertures 10 mm; for descriptions of River Imsa, see "Migration" in Material and methods section in online supplementary data[1]). 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 (24 May 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 that took place between the beginning of April and the end of May 2013. (For detailed information on wild smolt migration 2013, see Fig. S3[1].) All fish were released at the same time (1300–1315 h, water temperature: 11.3 °C, water velocity: 3.53 m^{3}·s^{-1}; for detailed information on Imsa River water properties for spring 2013, see Fig. S4[1]), and the migration rate and success was monitored by catching the descending fish in the trap, which is emptied at least twice a day (0800 and 1500 h) all year round.

Data treatment and statistical analysis

All data

Assumptions regarding normality of residuals and homogeneous 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 linear mixed effects model (LMM) analysis the package "nlme" (Pinheiro et al. 2013) was applied, while analysis based on generalized linear mixed models (GLMMs) were performed by the package "lme4" (Bates et al. 2013).

Growth

Growth was analysed applying 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. (For further details, see "Growth" in Data treatment and statistical analysis section in online supplementary data[1].)

Plasma cortisol data

These data were analysed using stepwise simplifications of LMMs or generalized least square (GLS) models. (For further details on statistical models, see "Plasma cortisol" in Data treatment and statistical analysis section, as well as Table S2, in online supplementary data[1].) 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).

Shelter-seeking

Shelter-seeking behaviour was analysed using a binary logistic regression within the GLMM. The GLMM analyses started with a global model containing Shelter, Density, Month, and all their interactions, as well as Tank nested within Density × Shelter, and interaction added as a random effect block. To gain power, the global model was reduced by sequentially removing nonsignificant interaction terms. (For further details, see "Shelter seeking" in Data treatment and statistical analysis section in online supplementary data[1].)

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 GLMMs with a binomial probability distribution. (For further details, see sections "Growth" and "Fin deterioration, silvering index and migration" in Data treatment and statistical analyses section in online supplementary data[1].)

Results

Growth

As indicated by the overall size (Table S1[1]), 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: $L_2 = 1.0$, $p = 0.6$).

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 with the smaller individuals. 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 with 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 ($\beta = 0.55 \pm 0.18$ 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

Fig. 2. Individual length (A) and mass (B) growth from October to 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 ($n = 210$). [Colour online.]

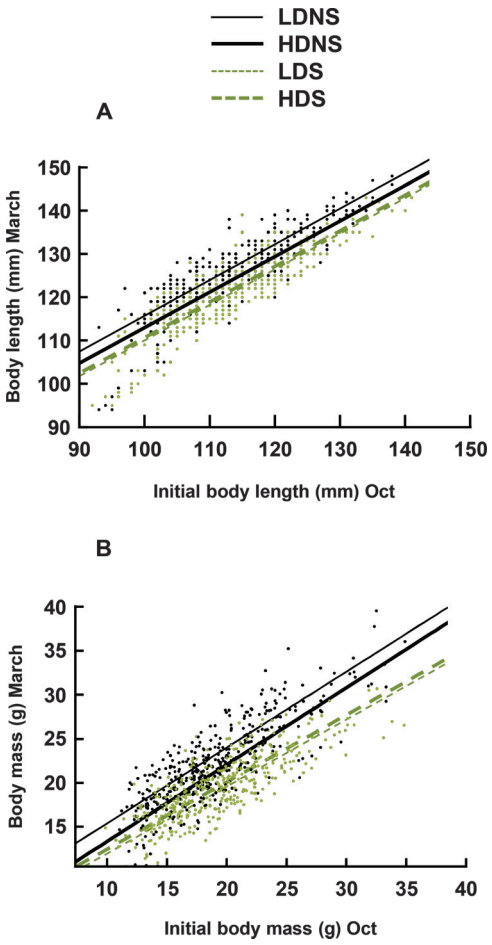


Fig. 3. Circulating plasma cortisol levels following human-induced in-tank disturbance (stress) compared with basal levels (basal) ($n = 18$). Values show means with 95% confidence intervals. Different letters indicate significant differences ($p < 0.05$).

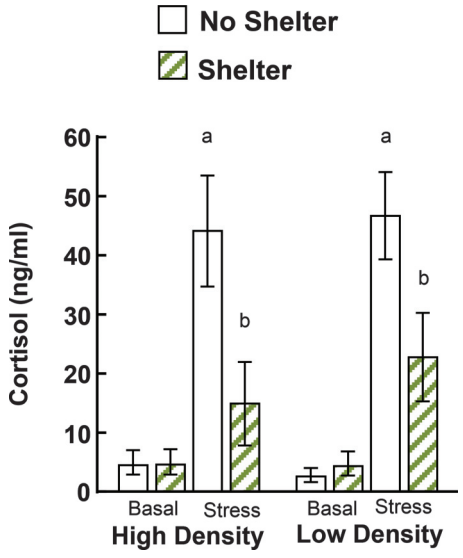
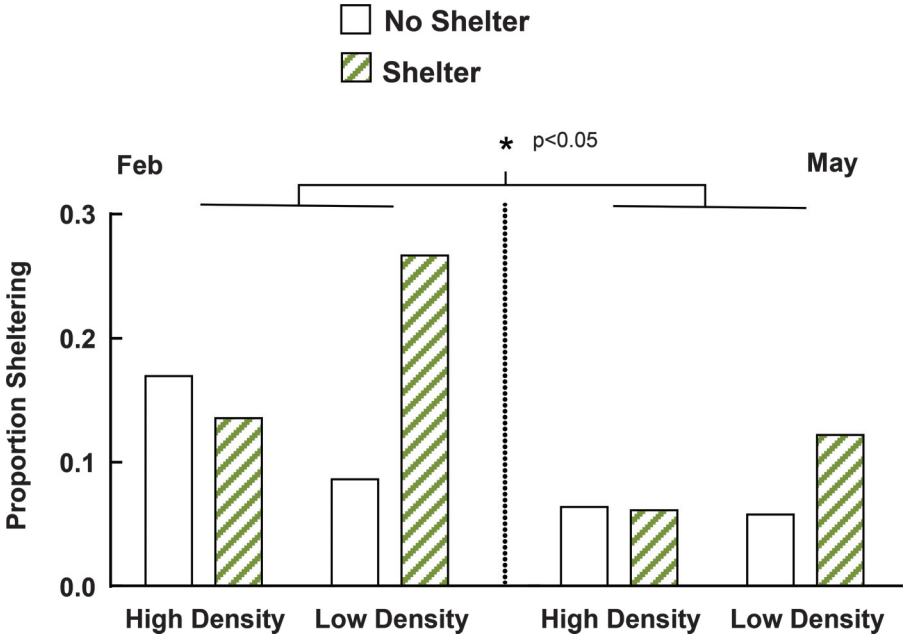


Fig. 4. Proportion of fish using shelter in a novel environment both as parr (February) and presmolts (May) (n = 60). The fish were placed in a shelter-seeking arena divided in two sections by a mesh with holes. The fish and the sheltering structures were placed on opposing sides, and shelter-seeking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



(Fig. S5[1]). 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 (unstressed <10 ng·mL^{-1}; 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 months, where fish in February (parr) sought shelter to a higher degree compared with fish in May (presmolts) ($F_{[1,441]}$ = 4.472, p = 0.035; for further details, see "Shelter seeking" in the Results section in online supplementary data[1]).

Intestinal barrier function

The TER of the intestine was lower in the High Density compared with the Low Density group, irrespective of intestinal region (proximal: L_1 = 9.7, p = 0.002; Fig. 5A; distal: L_1 = 15.0, $p < 0.001$; Fig. 5B). No significant difference in permeability for mannitol was found (Fig. S6[1]). For lysine uptake rate, there was an interaction effect in the proximal intestine (L_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: $L_1 > 8.8$, $p < 0.001$) and the High Density–No Shelter group having a higher absorption rate than the Low Density–Shelter group (post hoc test: L_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 with the Low Density group (L_1 = 10.9, p = 0.001; Fig. 5D).

Fin damage, smolt stage cortisol, and silvering index

For fin deterioration, there was an interaction effect (χ^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 or silvering index (Fig. S7[1]). Furthermore, no relation to body size was found (plasma cortisol: L_1 = 1.0, p = 0.3; silvering index: χ^2 = 0.13, p = 0.7).

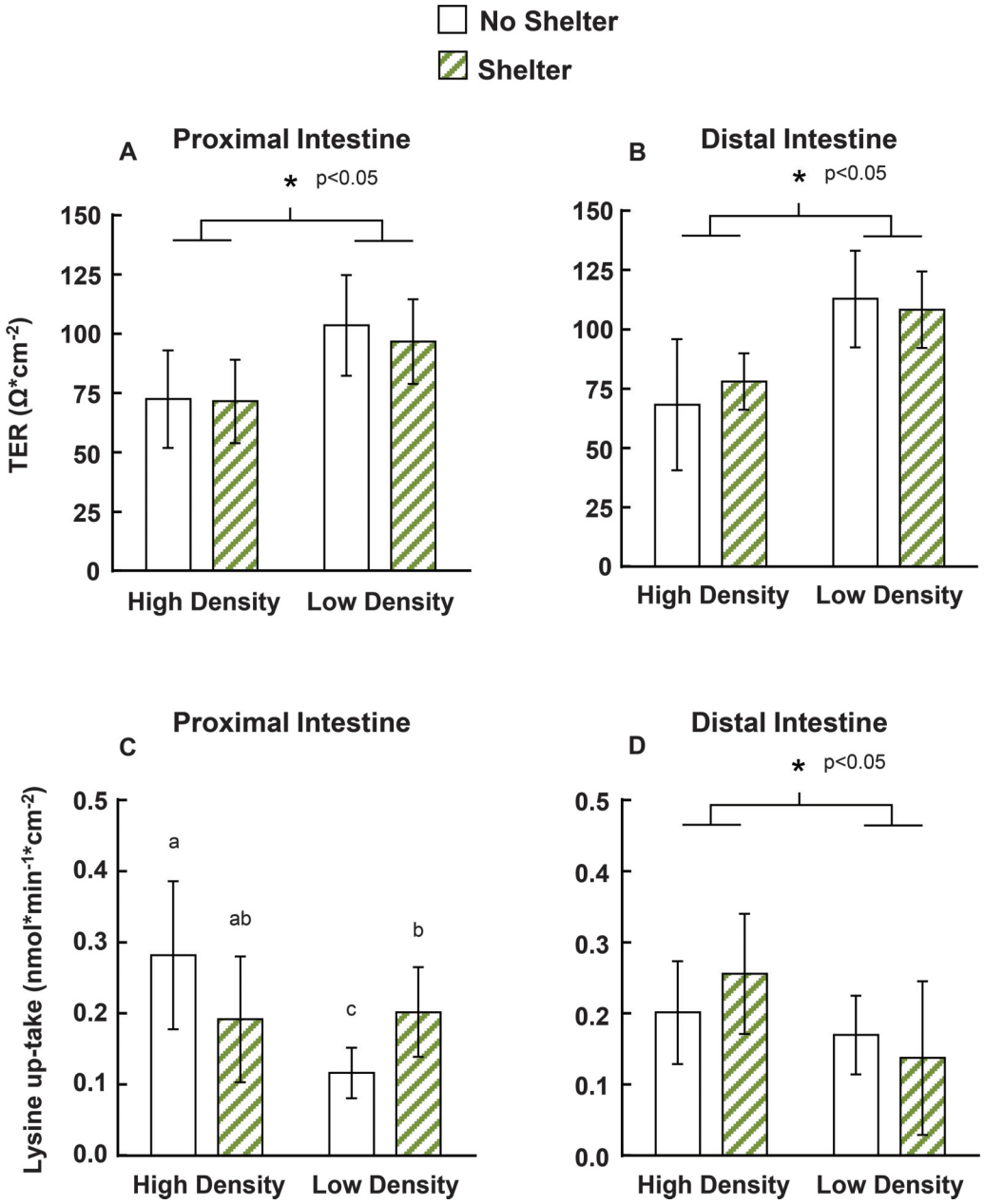
Migration

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; for further details on migration pattern, see Table S3[1]). Stepwise simplification of the full GLMM model with density, shelters, and individual body length as a covariate resulted in the only significant effects being body length (χ^2 = 13.96, $p < 0.001$) and shelter (χ^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 (χ^2 = 2.07, p = 0.15). There was a close to significant interaction effect of Density and Shelter (χ^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: four fish from High Density–No Shelter; four from High Density–Shelter; five from Low Density–No shelter; and two from Low Density–Shelter. This indicates that the majority of the fish that did not migrate in 2013 were probably killed by predation or for some reason did not seem to survive the following winter.

Discussion

The present study shows that changes to the captive environment can affect both physiological and behavioural traits connected to welfare and postrelease performance of Atlantic salmon. Compared with 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

Fig. 5. Intestinal barrier function measured through transepithelial resistance, TER (A, B) and intestinal nutritional uptake rate of the amino acid ^{3}H-lysine (C, D) as presmolts in May (n = 12). Bars show means, with error bars denoting 95% confidence intervals. Asterisk (*) and different letters indicate significant differences ($p < 0.05$).



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

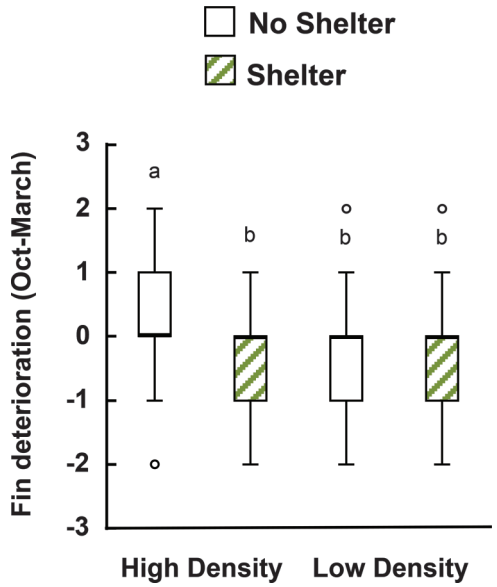
In January, an elevation of plasma cortisol above resting levels (Iwama 1998) was found in two out of three of the Low Density–No Shelter tanks; however, the overly large tank effects prevented detection of a statistical difference. The result is, however, in line with previous results from the same farming facility, where parr living at similar densities had higher resting cortisol levels in barren compared with 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 expe-

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 represents the second quartile (median). Whiskers represent the data within a 1.5 interquartile range, while dots represent data points outside the 1.5 interquartile range. Different letters indicate significant differences ($p < 0.05$).



rience 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 et al. 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 and that providing access to shelter should be considered when designing rearing environments.

Fin damage

Over winter (October–March), 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 (Turnbull 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 TER compared with 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 feedback 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, 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.

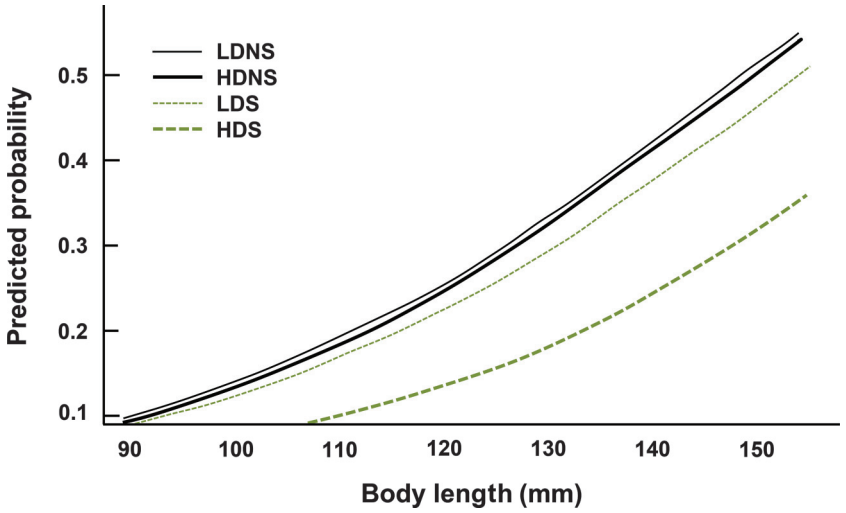
Growth and nutritional uptake

In contrast with 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 individuals (Brockmark et al. 2007). Enrichment structures restrict visibility, which can make it more difficult for dominant and larger individuals to monopolize food (Jobling 1985) and 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 (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 sug-

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_{LDNS} = 193, n_{HDNS} = 192, n_{LDS} = 151, n_{HDS} = 189). HD = high density, LD = low density, NS = no shelter, S = shelter. [Colour online.]



gests 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 postrelease 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 (M. Rosengren, 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 (M. Rosengren, 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, 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 with 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 seawater compared with 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 et al. 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 sheltering structures were combined 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 postrelease behaviour (Teixeira et al. 2007; Gaikwad et al. 2011). This has been shown in rearing environments 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 TER 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 postrelease 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, shows promising results, with significant or strong trends towards positive effects on intestinal barrier function, sheltering behaviour, stress hormone levels, and intraspecific 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 postrelease 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 to examine 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.

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