



Performance and welfare of Atlantic salmon smolt reared in recirculating or flow through aquaculture systems



Jelena Kolarevic^{a,*}, Grete Baeverfjord^a, Harald Takle^b, Elisabeth Ytteborg^b, Britt Kristin Megård Reiten^a, Sissel Nergård^c, Bendik Fyhn Terjesen^a

^a Nofima, NO-6600 Sunndalsøra, Norway

^b Nofima, NO-1432 Ås, Norway

^c Nofima, Ekkilsøy, NO-6530 Averøy, Norway

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ABSTRACT

The objective of this study was to compare growth performance, survival rate, physiological indicators and welfare of Atlantic salmon produced in a recirculation aquaculture system (RAS) or a flow-through (FT) system at identical temperature (13 °C), levels of dissolved oxygen (>85% saturation), hydraulic retention time (HRT) and water speed. In addition, the same parameters were followed up to four months after smolts seawater transfer. No significant difference in Atlantic salmon smolt growth performance was found during the production phase and up to four months after the transfer to sea cages. The cumulative mortality rate was within 1% prior to seawater transfer and at this point it was significantly higher for the RAS raised fish due to one week of increased mortality rate following vaccination. At the end of the experiment cumulative mortality was below 6% for both groups and did not differ between two groups. Although the growth performance has not reflected different production systems, several underlying differences on physiological and molecular level, as well as difference in welfare indicators were noted during the freshwater production phase. Atlantic salmon from the FT system showed a significantly higher mRNA expression of *NKAα1b* at the seawater transfer compared to the RAS produced smolts. On the other hand, *NKCC1*, *NKAα1a*, and *CFTR1* were significantly down-regulated during the photo-manipulation period (day 64) in Atlantic salmon from RAS and *NKAα1a*, and *CFTR1* were significantly upregulated in the same group after three days in seawater compared to the FT-raised smolts. The RAS raised fish were better to regulate plasma chloride levels early after commencement of the full light regime. In addition, partial pressure of carbon dioxide and concentration of bicarbonate in the whole blood of smolts produced in the FT system were significantly higher than in RAS produced smolts prior to sea water transfer. Prevalence of fin damage and shorter operculum were significantly higher among smolts produced in the FT system at the seawater transfer. The significant difference in fin damage between the two groups of fish remained after four months in the sea.

In total, 98% less water was used in RAS to produce Atlantic salmon smolt with similar performance and significantly lower prevalence of fin damage and operculum shortage versus more traditionally FT-system produced smolts, provided that the optimal conditions regarding stable temperature, tank conditions and water quality are met in FT-systems. This is the first experimental study that systematically compares the performance of Atlantic salmon smolt produced in environmentally controlled RAS and FT system during the freshwater phase and after transfer to sea cages.

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1. Introduction

Growing public demands for aquaculture sustainability and environmental concerns, together with the development of more advanced water treatment technology have led to an increased interest in water reuse systems for fish production. Contrary to the more traditional flow-through (FT) systems with a high consumption of new water,

the water treatment loop of recirculating aquaculture systems (RASs) allows for a 100-fold or more reduction in make-up water requirements (Roque d'Orbcastel et al., 2009a). With the reduction in water availability, RAS technology allows for increased smolt production to match the market demands. In addition, RAS may provide more stable and controlled environmental conditions for fish rearing, better disease management (Summerfelt et al., 2009a), enhanced biosecurity (Summerfelt et al., 2009b), reduction in carbon footprint related to fish transport and reduction in effluent load to meet the demands of increased environmental regulations (Martins et al., 2010).

* Corresponding author. Tel.: +47 93288322; fax: +47 71695301.
E-mail address: jelena.kolarevic@nofima.no (J. Kolarevic).

Production of Atlantic salmon smolt in RAS is increasing worldwide. In Europe the majority of 250 million Atlantic salmon smolts are produced yearly in Norway and Scotland in land-based FT farms (Bergheim et al., 2009). However, it was shown (Kittelsen et al., 2006) that the expected future increase in smolt production in Norway may be limited unless new water sources or production methods are used to reduce water requirements. In the last couple of years the aquaculture industry in Norway has increased the use of RAS technology and it is expected that 85 million smolts will be produced in systems with reused water by 2015 (del Campo et al., 2010). A full transition to RAS based smolt production occurred after year 2000 in the Faroe Islands (Bergheim et al., 2009). Consequently, larger smolts were produced in RAS presumably due to the better control of the water temperature during winter months. Moreover, Joensen (2008) reported a reduction in mortality rate from 20% to below 5% in the sea cages in the Faroe Islands. The experience from several commercial farms in Norway suggests that smolts produced in RAS show better production performances in terms of growth and/or survival in sea cages than smolts from FT systems (Ulgenes et al., 2008). However, there is a need to verify these observations in controlled experiments.

From a functional point of view, good fish welfare can be defined as the ability to maintain homeostasis and normal biological function, reflected in good health and absence of the disease, and with respect to aquaculture, good productivity (Segner et al., 2012). Stressors, such as changes in water quality observed in FT systems (Kristensen et al., 2009), can result in disruption of internal homeostasis which necessitates energy-demanding physiological adjustments known as allostasis (Segner et al., 2012). In order to facilitate the energy costs of acclimation a portion of the fish energy budget is reallocated from growth and reproduction (Wendelaar Bonga, 1997). It therefore seems likely that the stable RAS environment would impose a minimal allostatic load on the fish and promote more rapid growth.

The important part of the smoltification process in Atlantic salmon is the development of the salinity tolerance that is achieved through morphological changes in gills and increased transcription and translation of several enzymes, ion transporters and ion channels (Nilsen, 2007). Preparation for the parr-smolt transformation starts during the fresh water phase and is accompanied by an increase in gill Na^+ , K^+ -ATPase (NKA) activity (McCormick, 1995; McCormick and Saunders, 1987) and NKA α - and β -subunit mRNA levels (Seidelin et al., 2001). In addition, the Na^+ , K^+ , 2Cl^- co-transporter (NKCC) mRNA and protein levels and apical cystic fibrosis transmembrane conductance regulator I (CFTR I) mRNA levels increase during this period (Nilsen, 2007). The preparatory development of seawater tolerance in Atlantic salmon produced in RAS versus traditionally FT-system raised fish have not been studied so far.

The aim of this study was to test the hypothesis that Atlantic salmon smolts produced in RAS has better growth, survival rates and welfare compared to smolts produced in FT system at the same temperature, same levels of dissolved oxygen and same hydraulic retention time (HRT) and water speeds within the rearing tanks. Smolt physiological indicators were tested prior to the sea transfer and the expression of several branchial genes involved in osmoregulatory processes was examined. In addition, performance and survival rates of the Atlantic salmon smolts raised in the two different production systems were studied for four months after the transfer to sea.

2. Material and methods

2.1. Experimental animals

The freshwater stage of this study was done in the Nofima Centre for Recirculation in Aquaculture (NCRA) in Sunndalsøra (Terjesen et al., 2013). Atlantic salmon parr originating from the SalmoBreed Bolaks stain (Eikelandssosen, Norway), were reared in a FT system at Nofima

Sunndalsøra until average weight of $7.0 \pm 0.1 \text{ g}$ ($\pm \text{SD}$). The fish were stocked in eight 3.2 m^3 octagonal tanks (2230 individuals per tank) in June 2011. Four tanks were supplied with freshwater recirculating water, while the remaining four tanks received ground well water (FT). The same ground well water was used as a source of make-up water in the RAS. See Terjesen et al. (2013) for composition typical for this ground water well source. The fish were vaccinated during two days (days 76 and 77 of the experiment) at average weight of $61.4 \pm 2.7 \text{ g}$. After smoltification (at 109 days), 375 individuals ($92.2 \pm 3.8 \text{ g}$ average weight) from each tank were transferred to the sea and stocked in six randomly assigned 125 m^3 net pens, three net pens per treatment (500 individuals per net pen) at the Nofima seawater research station at Averøy for additional 167 days.

2.2. Experimental design

The RAS loop consisted of four octagonal tanks (3.2 m^3) with triple outlet configuration: particle trap in the tank center, the sludge collector outside the tank and the side wall drain (AquaOptima, Trondheim, Norway). Water from the tank outlets was treated with ozone prior to mechanical (Salsnes, Norway) and biological (Kaldnes MBBR with Biofilm Chip P, KrügerKaldnes, Sandefjord, Norway) treatment and degassing (AquaOptima). Further on, the water was oxygenated with downflow bubble contactors (AquaOptima) and then returned to the culture tanks (Fig. 1). Temperature and pH were continuously measured in the degasser sump, oxidation reduction potential (ORP) was recorded in the mechanical filter inlet and the recycled and make-up water flow rates and O_2 saturation at the tank level were also continuously monitored (Terjesen et al., 2013). Data from the probes were logged every fifth minute. The online pH probe was connected to a Walchem WDP 320 (Holliston, MA, U.S.) control system that was controlling addition of bicarbonate using a dosing system (IWAKI EW, Tokyo, Japan). The set pH point of 7.3 was chosen in order to maintain alkalinity levels around $50 \text{ mg L}^{-1} \text{ CaCO}_3$.

For the FT system (Fig. 2) ground well water with an average temperature between 6 and 9 °C was heated using a hot waste water source from a nearby aluminum plant (delivered at $85 \pm 3 \text{ °C}$, according to supplier), and a titanium heat exchange system (GEA Ecoflex, Sarstedt, Germany) to match the water temperature in RAS. The desired temperature in the FT system was maintained by a PLC (Siemens, Munich, Germany). The heat-exchanged water was subsequently degassed and oxygenated before entering the fish tanks.

Initial water flow in the tanks was set to 60 L min^{-1} and it was increased to 110 L min^{-1} (day 51) and finally to 135 L min^{-1} (day 85), to ensure good self-cleaning of the tanks, water quality and fish exercise (Jørgensen and Jobling, 1993). Water velocities were measured using a Höntzsch propeller with HLOG software (Waiblingen, German). Measurements were taken every 15 s during 7 minute intervals at 30 cm water depth and 65 cm from the chosen tank wall. Changes in the orientation of the inlet water pipe were made to achieve similar water speeds in replicate tanks from both systems.

After six weeks with continuous light (24L:0D), fish were subjected to a photoperiod with 12 h of light and 12 h of darkness (12L:12D) for six weeks, followed by six more weeks of 24L:0D, to induce smoltification. For vaccination, all fish were anesthetized with buffered MS 222 (Argent Chemical Laboratories, Redmond, WA, USA; buffered 1:1 with NaHCO_3) and injected with Alpha Ject 6-2 vaccine (Pharmaq, Oslo, Norway) during the last week of 12L:12D period. The osmoregulatory ability of the parr-smolts was tested in three consecutive 72 h saltwater challenge tests. Three weeks after 12L:12D period, ten fish from each treatment were transferred to a tank with static sea water (300 L) made by adding artificial sea-salt until 34‰ salinity. After 72 h, blood samples were collected from the caudal vessel using heparinized vacutainers (Terumo Europe, Belgium) and analyzed for plasma chloride (Kolarevic et al., 2013).

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