Aquaculture Research

Aquaculture Research, 2016, 1–19 doi: 10.1111/are.13121

Tank size and fish management history matters in experimental design

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Abstract

To investigate the questions: (1) does tank size affect fish performance; (2) does tank size prior to an experiment affect later fish performance and (3) how does performance in experimental tanks compare with rearing in industry-scale cages, Atlantic salmon smolts were acclimatized to 190, 3 or 0.9 m^3 tanks (Phase 1; 1.5 months) before redistributed to Phase 2 for 5 months. Question 1: fish in 190 m^3 tank were redistributed to 0.9 m^3 (190 m^3 → 0.9 m^3), 3 m^3 (190 m^3 → 3 m^3), or 103 m^3 (190 m^3 → 103 m^3) tanks. Question 2: fish in 0.9 m^3 tanks were redistributed to 3 m^3 tanks (0.9 m^3 → 3 m^3), and compared to fish from 190 m^3 tank (190 m^3 → 3 m^3). Question 3: fish were placed directly in 3 m^3 tanks, not moved (3 m^3 → 3 m^3), and compared to reference sea cages. Phase 2 mortality: 190 m^3 → 0.9 m^3 (46%), 190 m^3 → 3 m^3 (29%), 190 m^3 → 103 m^3 (19%), 3 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3 (<5%). Most mortality happened shortly after transfer. Our study suggests tank size dependent performance, based on growth and feed intake that increased with tank size. 190 m^3 → 103 m^3 fish were more active than 190 m^3 → 0.9 m^3 and 190 m^3 → 3 m^3 fish. 190 m^3 → 103 m^3 tanks had lowest relative variance. Previous tank scale history affected survival, since 0.9 m^3 → 3 m^3 fish showed higher survival than 190 m^3 → 3 m^3 fish. However, previous scale history did not affect growth rate, feed intake or somatic indexes. Fish performance in 3 m^3 → 3 m^3 tanks did not differ from the reference sea cages. However, fish in 103 m^3 tanks performed better than reference cages, suggesting potential for improved commercial production. This study demonstrates that management practices can influence fish performance and should be taken into account when designing experiments.

Keywords: tank size, experimental design, management, Atlantic salmon, performance, welfare

Introduction

The sustainable development of the Atlantic salmon industry is strongly dependent on research, and this has been the situation since the beginning of commercial production in the early 1970s. For better control, standardization, possibilities for replicated treatments and cost effectiveness, research is often done in small scale tanks, while the research customers, e.g. the salmon industry, request results that are directly transferable to much larger industry-scale units. The tanks and cages are getting larger, and the largest allowable cages today in Norway are 157 m in circumference (~45 000 m^3), which contrasts strongly with typical research tank sizes of, e.g. 0.5 m^3 volume. Since researchers are requested to provide useful data to the industry, there is a growing concern that the gap between the increasing sizes of industrial tanks or cages, and research units may affect the relevance of the scientific data, as this gap is believed to influence fish performance. A possible solution to increase the industrial relevance of the research data, and to minimize the effects of the scale gaps, is to simulate fish performance from data obtained in smaller tank sizes. Examples of modelling of aquaculture systems include the works of, e.g. Halachmi, Simon and Mozes (2014) and Anyadike, Mbajiorgu and Ajah (2015). The present paper will mention the work of M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark and B.F.Terjesen (submitted), where some data from the present experiment have been used for modelling fish performance in bigger units.

Another, more expensive solution, is to perform the experiments in commercial sized units.

Austreng, Storebakken and Åsgård (1987) published the first expected growth rate estimates for cultivated Atlantic salmon, and Åsgård, Holmefjord, Einen, Roem and Thodesen (1995) suggested to use growth models for salmon culture management. However, the salmon industry is rapidly developing, and commercial growth tables (% daily growth), are available (e.g. Skretting, 2011) smolts.

Regardless if research experiments are done by the same or different research groups, results may be difficult to compare and reproduce because the experiments are done with different designs, fish, seasons, protocols etc. Often this information is not easy to find in literature since it is seldom high-lighted in the publications, and therefore do not appear when performing literature searches. However, there are a few studies where comparisons of different experimental units are a topic. In a study by Jha, Barat and Nayak (2006) they found that koi carp *Cyprinus carpio* grew better and had higher survival rate in ponds compared to tanks. In another study, Boeuf and Gaignon (1989) found that Atlantic salmon smolts grew faster in larger (22.5 m^2) than in smaller (4 m^2) tanks when the fish were kept in the respective tanks for 5 months. In a third example, Ranta and Pirhonen (2006) found no differences in growth in rainbow trout *Oncorhynchus mykiss* that were raised in either 43 or 15 L tanks, but the fish consumed more feed in the larger tanks, suggesting reduced feed efficiency in the largest scale. The work by Ranta and Pirhonen (2006) differs from other similar studies in that they performed the experiment with single fish in each tank. These and similar studies show the difficulties that the industry face when receiving recommendations from researchers since the advice originate from different experimental designs and conditions. Hence, more knowledge is needed on how the experimental units themselves may affect the outcome of the research.

In the above mentioned examples of comparing different experiment unit scales and systems, the indicators survival, growth, feed intake, water velocity and flow were used to describe the conditions and effects. Often a variety of indicators are needed to reveal fish performance differences in different designs, and relevant indicators may additionally include behaviour (Takagi, Nashimoto, Yamamoto & Hiraishi 1993; Magellan, Johnson, Williamson, Richardson, Watt & Kaiser 2012), morphology and also differences in stress response (Latremouille 2003).

The aim of this study was to emphasize the importance of experimental design and management history, by investigating the effects of rearing tank size (scale), and different scaling histories on Atlantic salmon performance (mortality, growth, behaviour, physiology and morphology), and to compare with commercial production. Fish performance may depend on previous experiences; and scaling history in this case means that fish performances were evaluated after they had been acclimatized in one experimental scale size and then moved to another scale size.

Materials and methods

Experimental design

In this study, three research questions were investigated; (1) does tank size affect fish performance, (2) does tank size prior to an experiment affect later fish performance, and (3) how does performance in experimental tanks compare with rearing in industry-scale cages? To study these questions, two experimental phases (Phase 1–2) were used (Fig. 1).

In total, 12 896 Atlantic salmon smolts (from a larger commercial population; AquaGen strain QTL, Trondheim, Norway), were used in the study. The initial weight of the smolts were 72.1 ± 2.8 g (SD), and the fish were transported by a commercial fish transfer truck (seven tanks; 17 ppt) (Terje Malo Lastebiltransport, Molde, Norway) 400 km from SalMar Settefisk AS at Follasmolt to Nofima Centre for Recirculation in Aquaculture (NCRA) (Terjesen, Summerfelt, Nerland, Ulgenes, Fjæra, Megård Reiten, Selset, Kolarevic, Brunsvik, Bæverfjord, Takle, Kittelsen & Åsgård 2013) at Sunndalsøra in March 2012. Before transport, while still at the smolt farm, the fish were tested for smoltification by measuring gill ATPase activity and smolt index [a commercial smolt index from external evaluations; from 1 = no silvery skin colour, strong finger marks, no dark colour edges on the fins; to 4 = silvery skin colour, no finger marks, sharp dark colour edges on the fins (Pharmaq Analytiq, Bergen, Norway)]. Before transport, at the smolt farm, the fish came from commercial rearing tanks of 340 m^3. The entire experiment at NCRA was done in flow-through sea water.

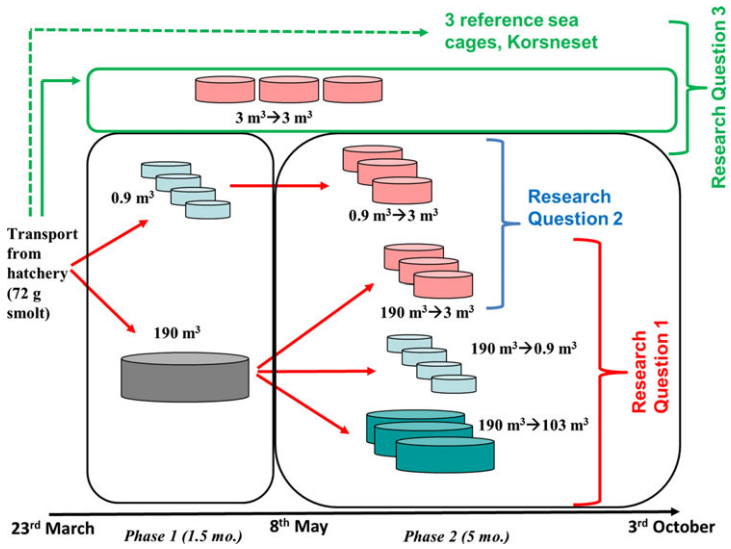

Figure 1 Experimental design of the 6.5-month long scale experiment. Approximately, 13 000 smolt were transported by truck to Nofima Centre for Recirculation in Aquaculture (NCRA), and placed in 0.9 m^3 (initial density 7 kg m^{-3}) or 190 m^3 (initial density 4.5 kg m^{-3}) tanks for 1.5 months (Phase 1), before being redistributed to new tank sizes (Phase 2) where they stayed for further 5 months. Initial density in Phase 2 was 3 kg m^{-3} in all tanks. All fish used in the experiment were of the same genetic origin. The experimental design was used to investigate three research questions: Research Question 1 = Does tank size at start of the experiment (Phase 2) affect the outcome of the experiment in terms of growth and survival? Treatments: 190 m^3 → 0.9 m^3, 190 m^3 → 3 m^3, 190 m^3 → 103 m^3. Research Question 2 = Does previous tank size (i.e. history) from Phase 1 affect later outcome of the experiment during Phase 2, on growth and survival? Treatments: 0.9 m^3 → 3 m^3, 190 m^3 → 3 m^3. Research Question 3 = How does rearing in experimental tanks compare with industry growth rates in cages? Treatments: 3 m^3 → 3 m^3 tanks, reference sea cages (M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F.Terjesen, submitted).

From the truck, at NCRA, the fish were randomly distributed between 0.9 m^3 ($N = 4$) and 190 m^3 ($N = 1$) tanks (Phase 1; 1.5 months). After Phase 1, the fish were redistributed to 103 m^3 ($N = 3$), 3 m^3 ($N = 6$) or 0.9 m^3 ($N = 4$) tanks (Phase 2; 5 months). Some fish were placed directly in 3 m^3 tanks ($N = 3$) from the truck, and not being further moved throughout the experiment. For further explanation of the experimental design, see Fig. 1 and the following text.

Phase 1, lasting for 1.5 months, served as an acclimation period where scaling history for the rest of the experiment was created. Initial stocking density in Phase 1 was 7 kg m^{-3} in each of the four 0.9 m^3 tanks, and 4.5 kg m^{-3} in the 190 m^3 tank ($N = 1$) (Fig. 1). The 2.5 kg m^{-3} difference in stocking density was necessary to have sufficient amount of fish in the smallest tanks to be able to sample. However, in a recent study on post-smolt stocking density, no significant effects of stocking density on performance or welfare was found up to 75 kg m^{-3} (S. Calabrese, T.O. Nilsen, L. Ebbesson, C. Pedrosa, S. Fivelstad, C. Hosfeld, S. Stefansson, B.F. Terjesen, H. Takle, C. Martins, H. Sveier, F. Mathisen, J. Kolarevic, A.K. Imsland & S.O. Handeland, in preparation).

The initial stocking density in all tanks in Phase 2 was 3 kg m^{-3}. Phase 2 lasted for 5 months, until October 2012. For individual identification, 20% of the fish were PIT-tagged (TracID AS, Stavanger, Norway) before transfer to the tanks in Phase 1. The PIT-tags contain individual identification, so when PIT-tagging fish prior to Phase 1, the Phase 2 tank to where the fish should be transferred is already decided. The PIT-tagging was performed prior to Phase 1 since it was necessary to know the fish ID also in Phase 1, and because predefinition of fish in Phase 2 tanks was wanted to avoid any unintentional selective distribution of fish in Phase 2. All fish were anesthetized (MS-222: Europharma, Leknes, Norway) before PIT-tagging and before transfer from Phase 1 to 2. The fish were fed *ad lib*, except using 10% above *ad lib* in periods with feed intake measurements (Helland, Grisdale-Helland &

Nerland 1996). The feed was produced by Ewos group (Tollbodalmenningen 1B, Bergen, Norway), using 3–7 mm pellets, of the types Adapt Marine 50, Opal 200, Alpha 500, and Alpha 200.

Experimental design related to research question 1

After Phase 1, the fish from the 190 m^3 tank were at the start of Phase 2 randomly distributed between 0.9 m^3 tanks ($N = 4$) (hereafter named 190 m^3 → 0.9 m^3), and 3 m^3 tanks ($N = 3$) (hereafter named 190 m^3 → 3 m^3) and 103 m^3 tanks ($N = 3$) (hereafter named 190 m^3 → 103 m^3) (Fig. 1).

Experimental design related to research question 2

After Phase 1, the fish from the four 0.9 m^3 tanks were moved to 3 m^3 tanks ($N = 3$) (hereafter named 0.9 m^3 → 3 m^3), and compared to fish transferred from the 190 to 3 m^3 tanks ($N = 3$) (hereafter named 190 m^3 → 3 m^3) (Fig. 1).

Experimental design related to research question 3

A parallel experiment was conducted in triplicate sea cages at Korsneset in Møre and Romsdal county in Norway (Latitude/longitude: 63°08′25″N/08°13′48″ Ø) ($N = 3$; 120 m in circumference, 17 000 m^3) (M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F.Terjesen, submitted) (Fig. 1). At the same time as the truck transferred approximately 13 000 smolts from Follasmolt to the tanks in Nofima Sunndalsøra (NCRA), a well-boat transported 600 000 smolts of the exact same genetic background and from the same population at Follasmolt to these three sea cages at Korsneset. As a parallel reference to this sea cage experiment at Korsneset, three 3 m^3 tanks were used (initial stocking density Phase 2 = 3 kg m^{-3}) in this study at NCRA. The fish were placed directly from the truck into these tanks, where they stayed for the entire experiment (Phase 1 and 2) without being further moved (Fig. 1). These reference tanks to the cage study (M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F. Terjesen, submitted) will hereafter be named 3 m^3 → 3 m^3. The main body of data from the cage part of the study is dealt with elsewhere (M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F. Terjesen, submitted), but since the design of the tank experiment strongly depended on the cage experiment, some data are also reported here (with permission). The cage and tank experiments were designed as similar as possible. In addition to the fish having the same genetic origin, the exact same feed batches were used. This was made possible by a previously arranged reporting system between the feed manufacturer and the project collaborators. Furthermore, similar feeding regimes and water temperatures were also used. Daily, water temperature data were transferred electronically from Korsneset to Nofima Sunndalsøra, which made it possible to regularly adjust the temperature in the tanks according to the prevailing cage temperature. Also photoperiod (hours L:D) were adjusted in the tanks according to the cage conditions.

Tank preparation and standardization

The following variables were standardized across treatments: All tanks were octagonal with the water inlet at the same side and the water outlet in the centre of the tank. As many measurements inside the tanks (e.g. lengths, distances, placements of light, feeders, inlet/outlet tubes etc.) as possible were done for all treatments and adjusted to standardize where possible. The depth of the tanks were (up to the water surface): 0.9 m^3 tanks (0.61 m); 3 m^3 tanks (0.89–0.99 m); 103 m^3 tanks (2.48–2.53 m) (variation due to the sloping tank bottom). Variables that were standardized between the tank scales were initial stocking density in Phase 2; feed type and feeding time frequency, also equal to the reference sea cages (equal feed type include the same batches and factory order numbers, in total 17 feed batches); light condition (358–408 lux at surface, following natural diurnal rhythm at the cage site); temperature [6.1 ± 0.9 (Phase 1) and 11.6 ± 2.0 (Phase 2)], similar and regularly adjusted to the reference sea cages by daily transfer of temperature data from the sea cages (measured at 5 m depths where, according to under water cameras, majority of the fish stayed). Oxygen saturation was controlled by oxygen sensors, programmable logic controllers and oxygenators for each tank (see Terjesen, Summerfelt *et al.* 2013), and adjusted not to go outside the range of 100–85% in outlet, in all tank scale treatments. In the period between March and May, the tanks were provided with feed three times a day, while this was reduced to twice a day between May and October, according to the reference cage site routines.

Since it was expected that water velocity was influenced by tank design (e.g. Klapsis & Burley

1984; Davidson & Summerfelt 2004; Neto, Zhu & Rajaratnam 2008) and fish movement (Rasmussen, Laursen, Craig & McLean 2005), the water flow and velocity were not standardized, and as a consequence, tank water hydraulic retention time (HRT) was unequal. Flow and velocity was set to typical values used for the three scales in research and industry, and monitored regularly during the experiment. This means that these variables increased with tank size, but not proportionally. In most setups of tanks in the salmon industry, HRT typically increase with tank size (Davidson & Summerfelt 2004), since the water velocity otherwise would increase to harmful levels for the fish, and the investment costs for water distribution technology would become extremely high. Feed distribution, as per cent of surface area covered, was expected to depend on water velocity and feeding systems, and this was monitored by measuring the degree of spreading from the feeders. In all cases, the feeding systems were adjusted to cover as large surface area of the tanks as possible. In the smaller 0.9 and 3 m^3 tanks, the feed were distributed using 120 mm wide belt feeders (Storvik Akva AS, Sundalsøra, Norway) and EX04 feeder with EX06 spreader (Poro AB, Kåge, Sweden) respectively. A computer-controlled pneumatic conveying system Akvasmart equipped with a rotor spreader (AKVA Group, Bryne, Norway), was used to distribute the feed in the larger 103 m^3 tanks.

Sampling, measurements and analyses

Mortality was recorded daily and is presented as % accumulated mortality. Individual weight and fork length, and bulk weights were measured at the beginning (March 2012) and at the end (October 2012) of the experiment. In addition, body weights were measured when moving the fish from Phase 1 to Phase 2 (May) and again in July. SGR (specific growth rate) was calculated from the formula:

$$\text{SGR (\%BW/day)} = ((\ln W2 - \ln W1) \times 100)/\text{days};$$

where $W1$ = start weight; $W2$ = end weight.

Thermal growth coefficient (TGC) was calculated from the formula:

$$\text{TGC} = \frac{\sqrt[3]{\text{end weight}} - \sqrt[3]{\text{start weight}}}{\text{sum of degree days}} \times 1000$$

Condition factor (CF) was calculated from the formula:

$$\text{CF} = (\text{weight (g)} \times 100)/\text{length (cm)}^3$$

Physiological stress variables were measured from blood at the beginning and at the day of terminating the experiment. Blood was analysed with i-STAT® portable clinical analyser (i-STAT; Abbott, Princeton, NY, USA) using CG8+ disposable cartridges, except lactate that was analysed with Arkray LactatePro test meter equipped with LactatePro Test Strips (Shiga, Japan). Blood samples were taken from the tail region (needle 22G × 1; 0.7 × 25 mm) into sodium fluoride/sodium heparin vacutainers, immediately after percussive stunning. After sampling, the fish were killed by a sharp stroke to the head. Immediately after sampling, the blood was transferred to i-STAT, analysing at 20°C. Blood gasses and pH were corrected for sea water temperature (Boutilier, Heming & Iwama 1984; Roth & Rotabakk 2012). When terminating the experiment, organs (heart, liver and gonads) were weighted for organo-somatic index. Also, all sampled fish were investigated for external welfare score, as described by Hoyle, Oidtmann, Ellis, Turnbull, North, Nikolaidis and Knowles (2007) and Kolarevic, Baeverfjord, Takle, Ytteborg, Reiten, Nergård and Terjesen (2014). Fin condition, cataract, skin lesions (wounds and scale loss), opercula damages and deviating colorations were evaluated and scored after a scale from 0 to 2; were 0 = not recorded; 1 = present; 2 = severely present.

The accuracy of i-STAT compared with traditionally laboratory analyses have been debated since the analyser originally is meant for human clinical testing (Harter, Shartau, Brauner & Farrell 2014). However, when compensated for temperature, i-STAT may successfully document relative differences between experimental groups (Cooke, Suski, Danylchuk, Danylchuk, Donaldson, Pullen, Bulté, O'Toole, Murchie, Koppelman, Shultz, Brooks & Goldberg 2008; Gallagher, Frick, Bushnell, Brill & Mandelman 2010). This also accounts for LactatePro (Cooke *et al.* 2008). For this study, the relative differences were important, and the minor deviations in accurate levels were considered acceptable.

Feed spill was collected according to Helland *et al.* (1996), and weighed on a daily basis during predefined periods of Phase 2; during the first

month (11th May–12th June), and thereafter during three one-weekly cycles (25th June–01st July; 06th–12th August; 06th–14th September) with 20 days without feed spill collection in-between cycles. A pellet water stability test was performed (Aas, Terjesen, Sigholt, Hillestad, Holm, Refstie, Baeverfjord, Rørvik, Sørensen, Oehme & Åsgård 2011) with feed samples from all feed batches used in the experiment to determine the % of dry matter (DM) in spill from all feed types. Representative feed spill types with average %DM content and those that varied by +1 or −1 SD were used for further calculation of feed DM (%). The recovery of DM in the feed spill (%) was further used to calculate accurate feed intake according to Helland *et al.* (1996) in the different tank scale treatments. Body weight specific feed intake (FI) (%/day) in each tank was thereafter calculated as:

$$\text{FI (\%/day)} = (\text{daily feed intake (g)} \times 100)/\text{biomass (g) at time},$$

where biomass in each tank was calculated as: $N \times$ Individual weight (g), where N represents the number of individuals in the tank, and the individual weight for the day in question was calculated using recorded TGC for periods between May–July and July–October, and daily recorded water temperatures in the experiment.

Swimming behaviour was recorded three times during Phase 2 (May, June and September) for research question 1. During each of these recordings, three video cameras (Go Pro, Hero 2, San Mateo, CA, USA) were divided between three tank sizes (0.9, 3 and 103 m^3). One camera was placed above each of the three tanks, enabling triplicate recordings and nine tanks filmed each time (tanks from the groups 190 $m^3 \rightarrow$ 0.9 m^3, 190 $m^3 \rightarrow$ 3 m^3, 190 $m^3 \rightarrow$ 103 m^3). Each time the tanks were filmed for 30 min each and later analysed for the relative amount of time (%) that the fish changed position in the tank (Espmark, Hjelde & Baeverfjord 2010). During each 30-min period, a standardized period of 10 min was selected and behaviours of 10 fish from three tanks of 0.9 m^3, 10 fish from three tanks of 3 m^3, and 15 fish from three tanks of 103 m^3 were analysed. Seawater used in all tanks during the experiment originated from the same source (Terjesen, Summerfelt *et al.* 2013). The following water quality parameters were measured in samples collected from the tank outlets during five occasions in Phase 2: pH, total ammonia nitrogen (TAN), total inorganic carbon (TIC) for calculation of carbon dioxide (CO_2; Terjesen, Summerfelt *et al.* 2013), turbidity in sea water, salinity and alkalinity. A portable Multi 3410 meter (WTW) with SenTix980 pH probe was used to measure water pH while the other water quality parameters were measured as described in Terjesen, Summerfelt *et al.* (2013). Temperature was logged continuously in the tanks using loggers (Ebro; Ebi 20 Ti, Germany). The loggers were placed inside the tanks and logged temperature every hour.

Water quality data for Phase 2 are given in Table 1. During the whole Phase 2, pH was significantly lower and CO_2 concentration was significantly higher in the 190 $m^3 \rightarrow$ 103 m^3 tanks compared to the water in any of the other tank scale treatment groups. During the three last measurements, in July, August and September, also TAN concentration was significantly higher in the 190 $m^3 \rightarrow$ 103 m^3 tanks, and during the two last measurements, in August and September, also water turbidity was higher in the same 190 $m^3 \rightarrow$ 103 m^3 group (Table 1).

Water velocity was measured at nine standardized points within all tanks during three occasions (May, June and August) (two occasions for 0.9 m^3 tanks) in Phase 2 using a Höntzsch propeller with HLOG software (Waiblingen, German). The nine measurement points with the measured velocities are shown in Table 2. Water flow was measured at start (May), in June and September. Water flow was determined by measuring water volume at the tank outlet pipes three times for 10 s and subsequently calculating the flow in L min^{-1}, except for the 103 m^3 tank treatment, in which a portable flow meter Portflow 300 was used (Micronics, Bucks, UK). Hydraulic retention time (HRT) was calculated as:

$$\text{HRT (min)} = \text{tank volume (L)}/\text{water flow (L min}^{-1}).$$

The averages velocities were: 0.9 m^3 tanks: 7 ± 3 cm s^{-1}; 3 m^3 tanks: 6 ± 4 cm s^{-1}; 103 m^3 tanks: 13 ± 4 cm s^{-1} (Table 2). Water flow did not increase proportionally to the tank volume: 0.9 m^3 tanks (17–24 L min^{-1}), 3 m^3 (54–71 L min^{-1}), 103 m^3 (790 L min^{-1}), giving Hydraulic Retention Time (HRT) in the 0.9 m^3 tanks of 37–55 min, in the 3 m^3 tanks 43–56 min, while HRT in the 103 m^3 tanks was 130 min.

Table 1 Water quality data, measured in the outlet water during five occasions in Phase 2

	Scale type				
	190 m^3 → 103 m^3	190 m^3 → 0.9 m^3	190 m^3 → 3 m^3	0.9 m^3 → 3 m^3	3 m^3 → 3 m^3
5th of June					
pH	7.63 ± 0.08[a]	7.84 ± 0.01[b]	7.81 ± 0.01[b]	7.79 ± 0.01[b]	7.79 ± 0.04[b]
Salinity (ppt)	34.4 ± 0.0	33.4 ± 0.0	33.4 ± 0.0	33.4 ± 0.0	33.73 ± 0.58
Turbidity (NTU)	0.44 ± 0.05	0.57 ± 0.1	0.5 ± 0.16	0.42 ± 0.04	0.4 ± 0.03
CO_2 (mg L^{-1})	1.81 ± 0.35[a]	1.15 ± 0.04[b]	1.24 ± 0.05[b]	1.36 ± 0.04[b]	1.34 ± 0.18[b]
TAN (mg L^{-1})	0.39 ± 0.07	0.27 ± 0.1	0.29 ± 0.04	0.31 ± 0.07	0.31 ± 0.03
Alkalinity (mg L^{-1})	110.67 ± 2.31	108.0 ± 6.53	106.67 ± 2.31	102.67 ± 6.11	104.0 ± 8.0
Temperature (°C)	11.45 ± 0.05[a]	11.45 ± 0.05[a]	11.40 ± 0.00[b]	11.40 ± 0.00[b]	11.20 ± 0.00[c]
31st of July					
pH	7.16 ± 0.09[a]	8.03 ± 0.01[b]	7.97 ± 0.0[bc]	7.93 ± 0.02[c]	7.93 ± 0.03[c]
Salinity (ppt)	33.5 ± 0.1	33.5 ± 0.0	33.47 ± 0.06	33.5 ± 0.0	33.5 ± 0.0
Turbidity (NTU)	0.48 ± 0.14	0.47 ± 0.1	0.65 ± 0.2	0.36 ± 0.05	0.39 ± 0.03
CO_2 (mg L^{-1})	6.99 ± 1.38[a]	0.75 ± 0.04[b]	0.86 ± 0.04[b]	0.96 ± 0.03[b]	0.95 ± 0.07[b]
TAN (mg L^{-1})	0.9 ± 0.16[a]	0.2 ± 0.02[b]	0.25 ± 0.01[b]	0.3 ± 0.02[b]	0.29 ± 0.01[b]
Alkalinity (mg L^{-1})	96.0 ± 5.29	99.5 ± 2.52	96.0 ± 4.0	92.0 ± 2.0	98.67 ± 4.62
Temperature (°C)	12.23 ± 0.20[ab]	12.25 ± 0.20[a]	12.14 ± 0.25[ab]	12.21 ± 0.26[ab]	12.05 ± 0.23[b]
15th of August					
pH	7.3 ± 0.03[a]	8.1 ± 0.01[b]	8.03 ± 0.02[c]	7.98 ± 0.02[c]	7.98 ± 0.03[c]
Salinity (ppt)	33.03 ± 0.76	33.2 ± 0.0	32.87 ± 0.58	33.43 ± 0.25	33.13 ± 0.12
Turbidity (NTU)	0.44 ± 0.07	0.46 ± 0.05	0.53 ± 0.27	0.37 ± 0.08	0.56 ± 0.07
CO_2 (mg L^{-1})	5.44 ± 0.39[a]	0.72 ± 0.03[b]	0.69 ± 0.11[b]	0.85 ± 0.05[b]	0.74 ± 0.26[b]
TAN (mg L^{-1})					
Alkalinity (mg L^{-1})	103.33 ± 5.77	100.0 ± 1.63	98.67 ± 1.15	98.0 ± 3.46	100.67 ± 1.15
Temperature (°C)	12.75 ± 0.07[a]	12.74 ± 0.08[a]	12.64 ± 0.09[b]	12.74 ± 0.09[a]	12.55 ± 0.08[c]
28th of August					
pH	6.96 ± 0.04[a]	8.03 ± 0.01[b]	7.95 ± 0.04[c]	7.89 ± 0.01[c]	7.89 ± 0.01[c]
Salinity (ppt)	34.0 ± 0.0	34.0 ± 0.0	34.0 ± 0.0	34.0 ± 0.0	34.0 ± 0.0
Turbidity (NTU)	0.57 ± 0.03[a]	0.43 ± 0.04[b]	0.38 ± 0.05[b]	0.36 ± 0.02[b]	0.35 ± 0.01[b]
CO_2 (mg L^{-1})	9.48 ± 1.07[a]	0.74 ± 0.02[b]	0.96 ± 0.1[b]	1.07 ± 0.03[b]	1.09 ± 0.03[b]
TAN (mg L^{-1})	0.78 ± 0.01[a]	0.21 ± 0.04[b]	0.26 ± 0.05[b]	0.28 ± 0.03[b]	0.27 ± 0.06[b]
Alkalinity (mg L^{-1})	106.67 ± 8.33	102.0 ± 3.65	105.33 ± 1.15	105.33 ± 6.43	99.33 ± 1.15
Temperature (°C)	14.29 ± 0.16[a]	14.19 ± 0.14[ab]	14.13 ± 0.12[bc]	14.22 ± 0.15[ab]	14.05 ± 0.14[c]
18th of September					
pH	6.77 ± 0.01[a]	7.9 ± 0.01[b]	7.86 ± 0.08[b]	7.69 ± 0.01[c]	7.75 ± 0.06[c]
Salinity (ppt)	32.0 ± 0.0	32.0 ± 0.0	32.0 ± 0.0	32.0 ± 0.0	32.0 ± 0.0
Turbidity (NTU)	0.55 ± 0.06[a]	0.28 ± 0.02[b]	0.27 ± 0.04[b]	0.32 ± 0.04[b]	0.3 ± 0.06[b]
CO_2 (mg L^{-1})	13.13 ± 0.48[a]	0.93 ± 0.07[b]	1.02 ± 0.2[b]	1.48 ± 0.14[b]	1.28 ± 0.16[b]
TAN (mg L^{-1})	1.12 ± 0.08[a]	0.19 ± 0.03[b]	0.26 ± 0.01[b]	0.28 ± 0.02[b]	0.24 ± 0.05[b]
Alkalinity (mg L^{-1})	100.0 ± 4.0	101.0 ± 2.0	100.67 ± 4.16	99.0 ± 1.41	103.33 ± 5.03
Temperature (°C)	13.70 ± 0.00[a]	13.60 ± 0.00[b]	13.50 ± 0.05[c]	13.60 ± 0.02[b]	13.50 ± 0.02[c]

Results are given when all groups are compared (ANOVA one-way), and are the same as when testing research question 1 (11 m → 7 m; 11 m → 1 m; 11 m → 2 m) with ANOVA one-way and research question 2 (190 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3) with *t*-test. Research question 3 (3 m^3 → 3 m^3 and sea cages) is not tested since results from sea cages are missing. Dissimilar letters define significant differences.

Statistics

Data were analysed with STATSDIRECT software (version 2.7.8). Comparisons between groups were tested with one-way ANOVA [and if significant, followed by multiple comparison tests (Tukey)], or unpaired *t*-test. For research question 1, comparisons were made between the treatments 190 m^3 → 103 m^3, 190 m^3 → 0.9 m^3, 190 m^3 → 3 m^3 and tested with ANOVA one-way; for Research question 2 comparisons were made between the treatments 190 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3 and tested with *t*-test. Research question 3 (3 m^3 → 3 m^3 compared to sea cages) were tested with *t*-test when data from sea cage were available. Differences in variances were tested

Table 2 Water velocities were measured at nine standardized measurement points in the tanks

Measurement points		0.3 m^3 tanks		3 m^3 tanks			103 m^3 tanks		
% Of tank depth	% Of tank width	2nd period (cm s^{-1})	3rd period (cm s^{-1})	1st period cm s^{-1})	2nd period (cm s^{-1})	3rd period (cm s^{-1})	1st period (cm s^{-1})	2nd period (cm s^{-1})	3rd period (cm s^{-1})
9	8	4	10	15	7	8		11	16
	18	2	9	14	6	7		9	17
	28	1	8	11	4	5		8	11
	38	1	6	12	4	3		6	7
35	10	4	8	14	7	8		11	16
	25	2	7	12	5	7		10	15
	35	1	4	10	3	4		7	12
65	10	3	7	13	6	8		11	15
	35	1	3	8	3	3	8	4	12

The measurement points are given in percentages of the tank depth and tank width. Water velocities are given in cm s^{-1}. Initial body length = 18.5 ± 1.2 cm; final body lengths = 42.3 ± 0.8 cm (190 m^3 → 103 m^3), 37.4 ± 1.1 cm (3 m^3 → 3 m^3), 37.7 ± 1.1 cm (0.9 m^3 → 3 m^3), 36.7 ± 0.4 cm (190 m^3 → 3 m^3), 33.9 ± 0.6 cm (190 m^3 → 0.9 m^3). Period 1 = May; Period 2 = June; Period 3 = August.

with Levene's test. To test if the weight variance in tank means differed between treatments, the % deviation for each tank mean from the relevant treatment mean was calculated, and the data subjected to arcsine square root transformation. All data are presented with means ± SD. The level of significance was set to 0.05, and the statistical units were defined as tanks.

This study was performed in accordance with Norwegian laws and regulations concerning experiments with live animals.

Results

Fish performance

Evaluations of smolt status before truck transport to NCRA showed that the fish were smoltified at the time of transport, as the smolt index was 3.7 (scale from 1 = not smoltified to 4 = complete smoltfied), and gill ATPase activity was 12.6 ± 2.4 μmol ADP mg^{-1} protein h^{-1} (Handeland, Wilkinson, Sveinsbø, McCormic & Stefansson 2004). At arrival to NCRA, the fish were in good condition; they had no skin lesions, but the fin conditions were impaired (1.6 ± 0.5 from the scale between 0 and 2; where 0 = no injuries; 2 = severe injuries). Blood analyses right after arrival indicated levels of stress as expected after truck transport (glucose = 7.3 ± 1.7 mmol L^{-1}, lactate = 2.5 ± 1.1 mmol L^{-1}) (Iversen, Finstad, McKinleyc, Eliassen, Carlsen & Evjen 2005).

During Phase 1, 4% of the fish transferred directly from the truck to 0.9 m^3 tanks died, while the numbers for 3 m^3 tanks and 190 m^3 tank were 3.5% and 2.3% respectively (Fig. 2a). For Phase 2, the highest mortality (46.3%) was recorded in the groups that were transferred from the 190 m^3 tanks in Phase 1 to the smallest 0.9 m^3 tanks in Phase 2 (190 m^3 → 0.9 m^3). Almost all mortality occurred within few days after transfer from Phase 1 to 2. The same timing of mortality was also the case for the groups that were transferred from the 190 m^3 tanks in Phase 1 to the 3 m^3 and 103 m^3 tanks in Phase 2 (190 m3 → 3 m3 and 190 m^3 → 103 m^3 groups respectively). The mortality for these groups was 28.8% and 19.2% respectively. The lowest mortality was recorded in the groups that experienced no change in scale (3 m^3 → 3 m^3: 3.5% mortality), and groups with a small change in scale (0.9 m^3 → 3 m^3: 2.7% mortality) (Fig. 2b). The three reference cages experienced between 6.5% and 9% accumulated mortality (M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F. Terjesen, submitted).

During the acclimation period in Phase 1, no growth rate differences were detected between the experimental groups, and at start of Phase 2, there were no differences in weight between any of the groups (190 m^3 → 103 m^3 = 84.26 ± 0.57 g; 190 m^3 → 3 m^3 = 86.34 ± 0.29 g; 190 m^3 →

0.9 m^3 = 81.91 ± 1.42 g; 3 m^3 → 3 m^3 = 85.89 ± 5.32 g; 0.9 m^3 → 3 m^3 = 86.93 ± 1.63 g; $F = 1.0$; $P = 0.4$). However during Phase 2, a growth gradient developed between the scales (190 m^3 → 103 m^3 > 3 m^3 → 3 m^3, 0.9 m^3 → 3 m^3, 190 m^3 → 3 m^3 > 190 m^3 → 0.9 m^3) (Fig. 3). Average end weights for Phase 2 are given in Fig. 3. In terms of growth rate, there were significant differences between the groups (SGR Phase 2: $F = 27.7$, $P < 0.0001$; TGC Phase 2: $F = 36.3$, $P < 0.0001$) (Fig. 3), indicating more rapid growth in the largest tanks, and clearly reduced growth when the fish were moved to the smallest tanks (190 m^3 → 0.9 m^3).

A Levine's test showed that there were no differences in variance between treatments, in terms of individual weight distribution within each tank. However, the relative variance (CV) based on the tank means within a scale treatment was the lowest in the 190 m^3 → 103 m^3 treatment, compared to all other treatments. There was a lower CV for weight between the 103 m^3 tank means (0.4%), compared to between the 0.9 m^3 tank means (8%), whereas the CV for the 3 m^3 tanks was 3.8%. A subsequent ANOVA on these data showed that the relative deviation was significantly lower between tanks in the 190 m^3 → 103 m^3 treatment, compared to the deviation between tanks in the 190 m^3 → 0.9 m^3 group ($F = 8.9$; $P = 0.002$).

The condition factor (CF), based on sampling at the day of termination of the experiment (October), was significantly lower for the 190 m^3 → 103 m^3 fish compared to all other group except for 0.9 m^3 → 3 m^3, whereas the other groups did not differ from each other (Table 3).

Initial stocking density in Phase 2 was equal in all tanks (3.01 ± 0.05 kg m^{-3}). However at the end of the experiment, significant differences in stocking density had developed ($F = 46.9$, $P < 0.0001$), as 190 m^3 → 103 m^3 (31.3 ±0.8 kg m^{-3}) = 3 m^3 → 3 m^3 (28.4 ± 0.9 kg m^{-3}) = 0.9 m^3 → 3 m^3 (27.4 ± 1.3 kg m^{-3}) > 190 m^3 → 3 m^3 (21.1 ± 1.5 kg m^{-3}) > 190 m^3 → 0.9 m^3 (12.3 ± 3.5 kg m^{-3}).

Feed intake

Feed intake (FI) (%BW/day) was measured during Phase 2 (Fig. 4). The highest feed intake was found among fish in the 190 m^3 → 103 m^3 tank scale treatment group in August, while fish in the 190 m^3 → 0.9 m^3 group had lowest feed intake during the same period ($F = 31.1$, $P < 0.0001$). Also in May–June, the 190 m^3 → 0.9 m^3 fish had lowest feed intake compared to all other groups ($F = 15.9$, $P < 0.001$) (Fig. 4).

Physiology, morphology and external welfare score

At the day of termination of the experiment in October, fish were sampled for physiological, morphological and external welfare score analyses. Table 3 summarizes the blood physiological analyses. For both pCO_2 and HCO_3^-, fish from the 190 m^3 → 103 m^3 group had significantly higher concentrations, while fish from the groups 3 m^3 → 3 m^3 and 190 m^3 → 0.9 m^3 had lower pH in blood compared to the 190 m^3 → 103 m^3 fish (Table 3).

Cardio somatic index (CSI), hepatic somatic index (HSI) and gonadal somatic index (GSI) are shown in Fig. 5. Fish sampled from the 190 m^3 → 103 m^3 group had significantly higher CSI compared to all other tank groups ($F = 13.8$, $P = 0.0003$), while the sea cage CSI was higher. The females from the 190 m^3 → 103 m^3 group had significantly smaller GSI compared to 190 m^3 → 0.9 m^3, 190 m^3 → 3 m^3 and 3 m^3 → 3 m^3 groups ($F = 7.3$, $P = 0.004$) (Fig. 5). No sexual maturation was observed in any of the treatments, based on gonadal indexes and visual inspections.

External welfare score of fin condition, cataract, skin lesions, gill damages and colouration from a scale (0 = nothing observed; 1 = some deviations; 2 = severe deviations) at day of termination revealed that many fish suffered from some fin damages (mainly dorsal fin) (average 1.2 ± 0.1) and deviating colouration (average 0.4 ± 0.3), but there were no significant differences between the treatment groups. One fish in the 0.9 m^3 → 3 m^3 suffered from cataract, and one fish in each of the 190 m^3 → 103 m^3 and 190 m^3 → 0.9 m^3 groups suffered from shortened opercula.

Behaviour

A quantification of fish swimming behaviour during three periods of the experiment (May, June and September) were done in triplicate for the scale treatments 0.9 m^3 (190 m^3 → 0.9 m^3), 3.0 m^3 (190 m^3 → 3 m^3), and 103 m^3 (190 m^3 → 103 m^3). The video recordings

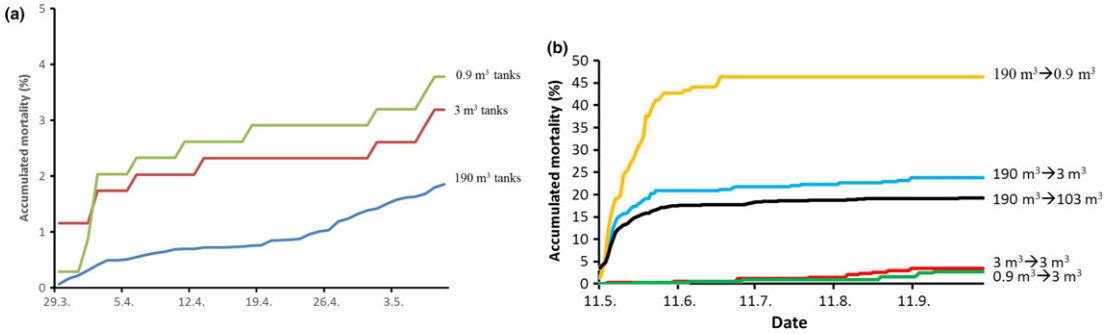

Figure 2 Accumulated mortality (%) during Phase 1 (a) and Phase 2 (b), Phase 1: 0.9 m^3 tank (green), 3 m^3 tank (red), 190 m^3 tank (blue). Phase 2: 190 m^3 → 0.9 m^3 (yellow), 190 m^3 → 3 m^3 (blue), 190 m^3 → 103 m^3 (black), 3 m^3 → 3 m^3 (red), 0.9 m^3 → 3 m^3 (green). Research question 1 (190 m^3 → 103 m^3; 190 m^3 → 0.9 m^3; 190 m^3 → 3 m^3), research question 2 (190 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3), and research question 3 [3 m^3 → 3 m^3 and reference sea cages (accumulated mortality 6.5–9%)] (M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F. Terjesen, submitted).

showed in general that fish in the 0.9 m^3 tanks swam with the same speed as the water velocity, with the result that the fish did not change their position in the tanks; some fish however drifted backwards. Fish in the 3 m^3 tanks also held their position against the current most of the time; however, they were more active compared to fish in the 0.9 m^3 tanks and some backwards drifting occurred. In the 103 m^3 tanks, the fish were far more active than in the previous two tank sizes. There were large individual variations in swimming behaviour, alternating between drifting, active swimming and holding position. This description of behaviour was also quantified. During all three periods with measurements, the fish in the 190 m^3 → 103 m^3 group changed position more often than any of the other groups (May: $F = 39.4$, $P < 0.0001$; June $F = 168.2$, $P < 0.0001$; September $F = 371.9$, $P < 0.0001$), and in September there was a dose–response relationship between scale and time that the fish spent in changing position (190 m^3 → 103 m^3 > 190 m^3 → 3 m^3 > 190 m^3 → 0.9 m^3) (Fig. 6).

Discussion

Design and dimensioning of research facilities vary, and tanks and equipment in research infrastructures normally differ from what is used in commercial farms where the results from the studies often are intended to be used. Also, for research it is more feasible to run less costly experiments in smaller tanks since this allows for more replicates and experiments. However, this study demonstrates that the size of the tanks where the fish are reared, and also if the fish are moved between tanks of different sizes, matters for their performance.

In this study, we used the experimental design shown in Fig. 1 to answer the three research questions; (1) does tank size affect fish performance, (2) does tank size prior to an experiment affect later fish performance and (3) how does performance in experimental tanks compare with rearing in industry-scale cages? The three research questions are visualized in Fig. 1. In the following discussion the three research questions will be dealt with. In addition, the experimental design is suitable to include all the groups into the discussion of effects of tank size and management history, and how the different tanks are suitable to achieve expected growth.

As already described, some experimental variables, that depend on the tank size *per se*, were intentionally not standardised, but monitored since they are direct consequences of increased scales (Davidson & Summerfelt 2004). For the same reason, fish densities were not continuously equalised, although the initial stocking density in Phase 2 was the same in all tank scales. The significance of tank design has previously been described in a review by Klapsis and Burley (1984) where they reviewed the influence of water flow on feed and temperature distribution in different tank designs.

During Phase 1, the mortalities in all tanks (190, 3 and 0.9 m^3) were low, and did not differ.

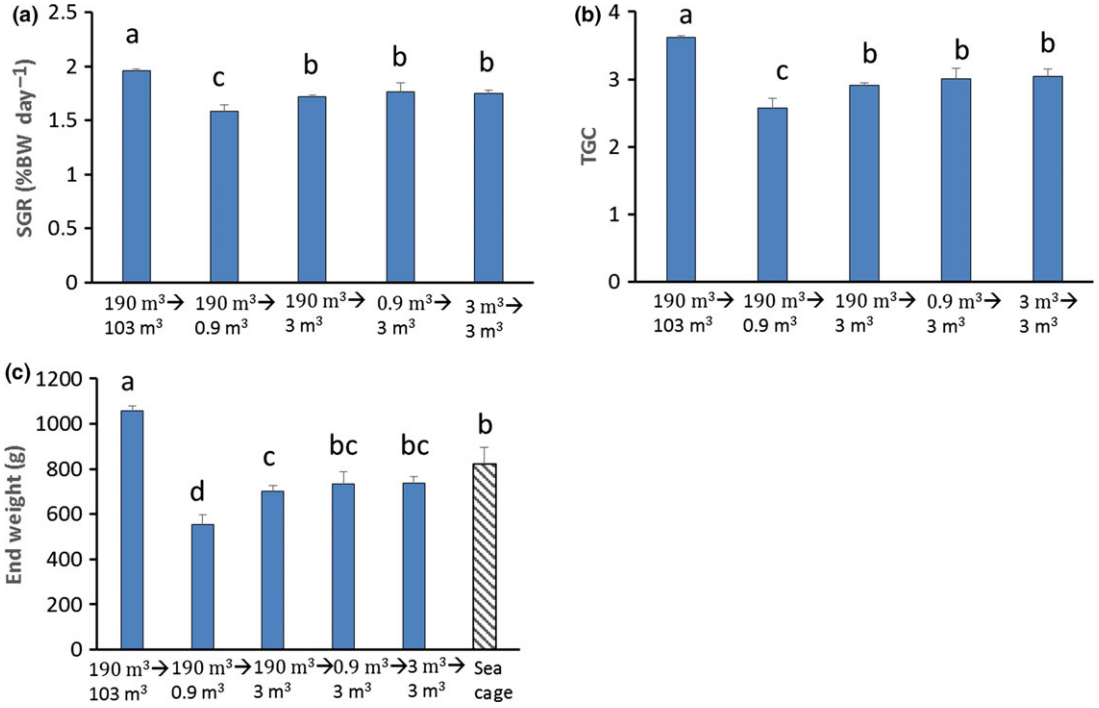

Figure 3 Specific growth rate (SGR) (a), thermal growth coefficient (TGC) (b) and end weight (g) (c) for Phase 2 ($N = 3$ for all groups, except 190 m^3 → 0.9 m^3 where $N = 4$ tanks). Dissimilar letters define significant differences. Results are given when all groups are compared (ANOVA one-way), and are the same as when testing research question 1 (190 m^3 → 103 m^3; 190 m^3 → 0.9 m^3; 190 m^3 → 3 m^3) with ANOVA one-way and research question 2 (190 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3) with *t*-test. Research question 3 (3 m^3 → 3 m^3 and sea cages) is only tested for end weight since data for SGR and TGC for the same period (Phase 2) is not available.

Table 3 Physiological analyses ($N = 3$) and condition factor at day of termination of the experiment in October 2012

	Scale name				
Variable	**190 m^3 → 103 m^3**	**190 m^3 → 0.9 m^3**	**190 m^3 → 3 m^3**	**0.9 m^3 → 3 m^3**	**3 m^3 → 3 m^3**
pH	7.5 ± 0.0^a	7.3 ± 0.1^b	7.4 ± 0.1^{ab}	7.4 ± 0.0^{ab}	7.3 ± 0.1^b
pCO_2 (mmHg)	22.6 ± 2.4^a	10.9 ± 3.2^b	10.1 ± 1.8^b	10.8 ± 2.6^b	11.7 ± 6.0^b
pO_2 (mmHg)	22.5 ± 2.2	24.6 ± 6.7	23.6 ± 1.5	22.3 ± 5.5	19.5 ± 7.0
HCO_3 (mmol L^{-1})	32.1 ± 2.3^a	9.0 ± 0.4^b	10.5 ± 0.6^b	11.1 ± 0.2^b	10.3 ± 0.4^b
Glucose (mmol L^{-1})	3.9 ± 0.4	3.9 ± 0.2	4.0 ± 0.5	4.1 ± 0.4	4.1 ± 0.4
Lactate (mmol L^{-1})	5.2 ± 0.9	4.7 ± 0.6	3.8 ± 0.8	4.1 ± 0.5	4.5 ± 1.3
Condition factor	1.34 ± 0.0^a	1.4 ± 0.0^b	1.41 ± 0.0^b	1.38 ± 0.0^{ab}	1.4 ± 0.0^b

Analyses of blood gasses are performed with i-STAT portable instrument and corrected for water temperature (12.8 °C). Results are given when all groups are compared (ANOVA one-way), and are the same as when testing research question 1 (190 m^3 → 103 m^3; 190 m^3 → 0.9 m^3; 190 m^3 → 3 m^3) with ANOVA one-way and research question 2 (190 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3) with *t*-test. Research question 3 (3 m^3 → 3 m^3 and sea cages) is not tested since results from sea cages are missing. Dissimilar letters define significant differences.

The low increased mortality during phase 1 is as expected after inset, however, the slightly higher mortality in 0.9 m^3 tanks (Fig. 2a) suggests a tank effect already here. Approximately 1 week after transfer of fish from the 190 m^3 tank in Phase 1 to the three different tank sizes in Phase 2, some mortality was recorded. The mortality in Phase 2 was particularly high among fish that were moved between tanks with large differences in scales, e.g. to the 0.9 m^3 tanks

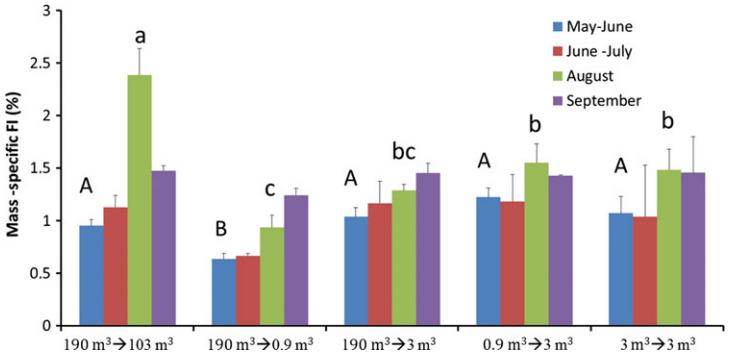

Figure 4 Mass specific feed intake (%BW/day) was measured four times during Phase 2 in the experiment, ranging from the start of Phase 2 in May and until September, 1 month before terminating the experiment ($N = 3$ for all groups, except 190 m^3 → 0.9 m^3 where $N = 4$). Results are given when all groups are compared (ANOVA one-way), and are the same as when testing research question 1 (11 m → 7 m; 11 m → 1 m; 11 m → 2 m) with ANOVA one-way and research question 2 (190 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3) with *t*-test. Research question 3 (3 m^3 → 3 m^3 and sea cages) was not tested since feed spill was not measured in the cages, hence feed intake here is overestimated. Different letters define significant differences between treatments.

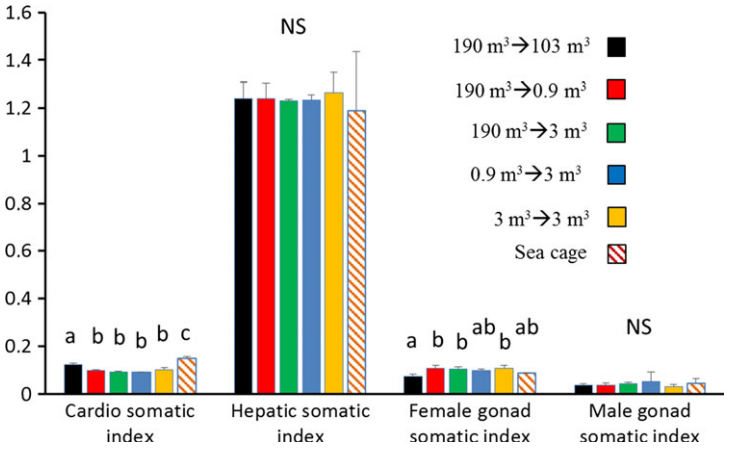

Figure 5 Cardio somatic index (CSI), hepatic somatic index (HSI) and gonado somatic index (GSI) at the day of termination of the experiment. Dissimilar letters define significant differences. Results are given when all groups are compared (ANOVA one-way), and are the same as when testing research question 1 (190 m^3 → 103 m^3; 190 m^3 → 0.9 m^3; 190 m^3 → 3 m^3) with ANOVA one-way and research question 2 (190 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3) and 3 (3 m^3 → 3 m^3 and sea cages) with *t*-test.

(190 m^3 → 0.9 m^3), but also among fish moved to the 3 m^3 (190 m^3 → 3 m^3) and 103 m^3 (190 m^3 → 103 m^3) (research question 1) tanks some mortality was observed. The mortality was however low among fish that were kept in 3 m^3 tanks throughout the entire experiment (3 m^3 → 3 m^3 research question 3), and among fish that were moved between tanks with small size differences, i.e. 0.9 m^3 → 3 m^3 tanks (Fig. 2). Most likely, the handling (i.e. netting, PIT-tag reading, exposure to air during transfer) during transfer from the 190 m^3 tank accelerated the mortality. Although the handling did not exceed normal practice and the fish were anesthetized before transfer from Phase 1 to 2, it is possible that the fish were sensitive towards handling 1.5 months after 'sea transfer'. Commercially, farmed fish are normally not handled so shortly after sea transfer. A veterinary report concluded that the mortality was not pathological, but a cause of handling of the fish (*pers com*. Kystlab 15th of May 2012, Grunde Heggland). The mortality was higher when the fish were transferred 190 m^3 → 0.9 m^3 compared to when the fish

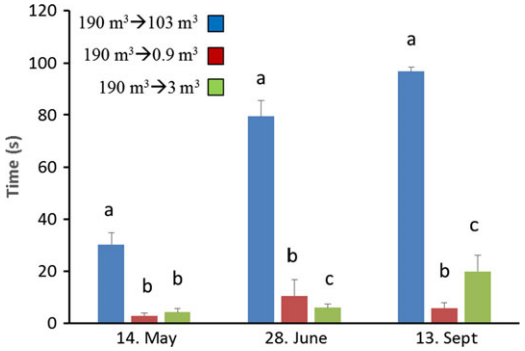

Figure 6 Swimming behaviour was video recorded three times during Phase 2 (research question 1). The video recordings were analysed for time (s) that the fish changed position in the tank. Each bar represents observations on 10 fish from triplicate tanks (total 30 fish) (190 m^3 → 0.9 m^3 and 190 m^3 → 3 m^3), and 15 fish from triplicate tanks (total of 45 fish) of the 190 m^3 → 103 m^3 scale. Dissimilar letters define significant differences within each date.

were moved 190 m^3 → 3 m^3 and 190 m^3 → 103 m^3 (research question 1). Also, the mortality when moved 190 m^3 → 3 m^3 was higher than 0.9 m^3 → 3 m^3 (research question 2). The reasons for these findings are not known, but it is proposed that the transfer from the larger tanks to much smaller tanks may have caused too much stress to the fish due to the big change in tank size. Approximately 2 weeks after transfer to Phase 2, the mortality almost ceased, most likely because by then the systems were stabilized and the lethal effects of transfer had stopped. No particular mortality was observed when transferring fish from the large commercial tanks at the smolt farm to the smaller tanks in Phase 1. This may be because the conditions during the truck transport were relatively good, and that the fish did not experience too much stress during transport. Also, the scale difference between the truck (2.4 m^3) and the 0.9 m^3 was smaller than for 190 m^3 → 0.9 m^3. Finally, it may be that the smolts were in a less sensitive stage during truck transport than later during transfer between tanks. Estudillo, Duray and Marasigan (1998) also experienced higher mortality among milkfish *Chanos chanos* larvae in smaller tanks, and they discussed the dilemma that even though it is evident that the survival is better in large tanks, researchers continue to use small tanks due to lower operational costs.

In May, just before transfer from Phase 1 to Phase 2, the dead fish in the 190 m^3 tank were smaller ($t = 4.9$; $P < 0.0001$) than the alive fish in the same tank sampled in the same period. However, the remaining fish in the 190 m^3 → 0.9 m^3 tanks, were the mortality was highest, showed the lowest growth rate throughout the experiment, suggesting that a possible size-selective mortality in Phase 1 was not consequent throughout the experiment. Other publications, mostly on wild fish populations, have shown both the existence and the absence of size-selective mortality (e.g. Claiborne, Fisher, Hayes & Emmett 2011; Claiborne, Miller, Weitkamp, Teel & Emmett 2014). Also, Solberg, Zhang, Nilsen and Glover (2013) showed that the relative difference in body weight between different salmon groups decreased with increasing mortality, thus suggesting that size-selective mortality may reduce growth differences.

In Phase 1, no differences in body weight were detected between fish in 0.9 and 190 m^3 tanks. Low temperatures during Phase 1 (range 4.9–8.1°C; TGC = 0.92 ± 0.05) may explain why body weight differences were not detected. The relatively low SGR in Phase 1 also suggest little growth in general and thus the weight difference had not yet been established.

The results from this study demonstrate that tank scale do affect growth and performance (research question 1). At start of Phase 2, in May, there were no differences in fish weight between the Phase 2 tanks. However, during Phase 2 the growth started to deviate between the tank sizes. From July to October, a pattern developed that resulted in significantly increased SGR and TGC with increasing scale (190 m^3 → 103 m^3 > ;190 m^3 → 3 m^3 > 190 m^3 → 0.9 m^3). Initial stocking density was the same in all tanks while the end density in the 190 m^3 → 103 m^3 tanks was higher than in the 190 m^3 → 0.9 m^3 tanks, as a consequence of the growth rate differences. This result suggests that the conditions in the smallest tanks (0.9 m^3) after transfer were not optimal and resulted in less feed intake, poor growth and hence low final biomass. Other studies have also shown a positive relationship between increased tank size and growth (Boeuf & Gaignon 1989; Wexler, Scholey, Olson, Margulies, Nakazawa & Suter 2003). However, in a study by Ranta and Pirhonen (2006), the authors found no difference in growth among juvenile rainbow trout *Oncorhynchus mykiss* raised in two different tank sizes even though the fish

in the bigger tanks had a higher feed intake; that is reduced feed efficiency may have occurred in the larger tanks. In the study by Ranta and Pirhonen (2006), one fish was kept per tank. These findings support the idea that growth also depend on the interaction between individual fish (Ranta & Pirhonen 2006), and the tank environment created by other fish. Also Kirschbaum, Hensel and Williot (2006) failed to detect tank size effects on growth rate in European Atlantic sturgeon *Acipenser sturio* L. They showed higher SGR among fish raised in 11.6 m^3 tanks and smaller initial densities compared to 6.8 m^3 tanks with higher initial densities. At the same initial densities, the SGR were similar between the different tank sizes, thus indicating density effect and no tank size effect (Kirschbaum *et al.* 2006). Austreng *et al.* (1987) indicated expected SGR = 0.7–0.8 for comparable fish size and temperature as Phase 1 (SGR = 0.4). This support that all groups in Phase 1 had some retarded growth. In Phase 2, the most rapid growing fish group (190 m^3 → 103 m^3) had SGR = 2.0, which is well above the 1.2–1.3 expected from Austreng *et al.* (1987). The TGC = 3.5 among the 190 m^3 → 103 m^3 fish in Phase 2 is comparable to the TGC = 3.5 reported for grow-out in commercial farms (Holmefjord, Åsgård, Einen, Thodesen & Roen 1994). Also, according to present commercial calculations of expected growth (e.g. Skretting 2011), the fish in the experiment, taken into account the relevant temperature and fish size, were expected to achieve end weight of 1060 g. This is approximately equal to the 190 m^3 → 103 m^3 fish (end weight = 1057 ± 20.8 g). This indicates that the growth performance achieved in our 190 m^3 → 103 m^3 tanks is relevant to the industry.

Results from weight distribution in the tanks showed that there were significant differences in individual weight variance between tank means (research question 1, effects of tank scale). The lower relative variance (CV) in weight between the 103 m^3 tank means (0.4%), compared to the 0.9 m^3 tank means (8%), may be influenced by a number of factors, such as complex relationships between water velocities, feed distribution and fish performance, size and behaviour, and need to be further investigated. However, a lower variance between statistical units (tanks) in an experiment, will improve detectability of smaller differences using the same number of replicates, that is low variance improves statistical power. Hence, this study indicates that an improved power will result when running experiments with Atlantic postsmolt in larger tanks (103 m^3), compared to small (0.9 m^3), when final body weight is the target variable.

To evaluate growth rates, factors such as feed availability, feed intake, water quality, water velocity, and HRT and fish behaviour need to be taken into account. Mass specific feed intake (FI) (Fig. 4) showed that the 190 m^3 → 103 m^3 group had higher FI in August compared to the other groups, and the 190 m^3 → 0.9 m^3 group had lower FI than all other groups in May–June and lower than the 190 m^3 → 103 m^3 groups in August. Farmed salmon eat more easily pellets that are in the water column than pellets lying on the tank bottom. The higher FI and growth rate in large tanks may be partly explained by the longer time it takes for the pellets to reach the bottom compared to the situation in small tanks, thus enabling the fish to feed for a longer time. However, it is likely that the differences in FI quite early in the experiment only partly explain the large differences in growth. Water velocity was highest in the 190 m^3 → 103 m^3 group and lowest in the 190 m^3 → 0.9 m^3 group. In pre-smolts of Atlantic salmon, several studies have shown that relatively high water velocities improve growth rate and disease resistance (e.g. Castro, Grisdale-Helland, Helland, Kristensen, Jørgensen, Helgerud, Claireaux, Farrell, Krasnov & Takle 2011). Recently, preliminary results indicate that higher water velocity also improves growth rate in Atlantic salmon postsmolts reared in RAS (Terjesen, Ytrestøyl, Kolarevic, Calabrese, Rosseland, Teien, Åtland, Nilsen, Stefansson, Handeland & Takle 2013). Also in sea trout *Salmo trutta*, Bugeon, Lefevre and Fauconneau (2003) showed better growth when the fish were subjected to higher water velocity. In another study by Hafs, Mazik, Kenney and Silverstein (2012) the authors did not find any influence on growth by velocity in rainbow trout in an almost 3 month long experiment. However, the recent high number of studies regarding exercise in Atlantic salmon (reviewed in Takle & Castro 2013), lead us to propose that the improved growth rate in the 190 m^3 → 103 m^3 treatment was partly due to the beneficial effects of higher water velocity. Increased water velocity is often an observed consequence of using larger tanks, even at higher HRTs (Davidson & Summerfelt 2004).

The differences in water velocity may also have been reflected in fish behaviour during this study, expressed as time spent changing position (Research question 1). Fish in the 190 m^3 → 103 m^3 group changed position more often compared to the 190 m^3 → 0.9 m^3 group that mostly did not change position at all, but instead kept position against the current. When water velocity increased, also the frequency of changing position increased. Other authors have also used orientation towards current and agonistic behaviour to describe the behaviour in tanks (Ross, Watten, Krise, Dilauro & Soderberg 1995; Ross & Watten 1998). In this study, it is believed that in the small 0.9 m^3 tanks the lower, but present velocity created an approximate homogeneous environment, and was sufficient to allow the fish to orient themselves quite stable against the water current. The fish in the smallest tanks only changed position during agonistic behaviour or when they recaptured their position after being chased away. In the 103 m^3 tanks, however, the higher water velocity associated with the larger tank volume may have created a heterogenic hydrodynamic environment and thus varying swimming behaviour related to the position of the fish (standing against the current, swimming actively backwards, swimming with the current). The highest stocking density obtained (31.3 ± 0.8 kg m^{-3} in the 103 m^3 tanks) still gives the fish sufficient space to move freely if preferred (RSPCA, 2012). It is proposed that the more active fish in the 190 m^3 → 103 m^3 group resulted in fit fish with relatively larger hearts (Fig. 5) (Hinterleitner, Huber, Lackner & Wieser 1992; Dalziel & Schulte 2012; Takle & Castro 2013), slimmer body shape and lower condition factor (Table 3) (Claireaux, McKenzie, Genge, Chatelier, Aubin & Farrell 2005; McKenzie, Pedersen & Jokumsen 2007), and that additionally grew better (Davison 1997; Yogata & Oku 2000; Brown, Bruce, Pether & Herbert 2011). Increased swimming led to a reduced condition factor in the 190 m^3 → 103 m^3 group compared to the other groups, suggesting a reduced lipid deposition which may have improved feed intake and growth.

Water quality analyses in Phase 2 showed higher water CO_2 concentration and lower water pH in 190 m^3 → 103 m^3 tanks at all five sampling points during the experiment. It is likely that the increased levels of CO_2 and low pH is a consequence of increased biomass in the 190 m^3 → 103 m^3 group (Tang, Thorarensen, Brauner, Wood & Farrell 2009). It is also likely that the high CO_2 accompanied by low pH, and the higher total ammonia nitrogen (TAN) in the water correlated with the longer hydraulic retention time in the 190 m^3 → 103 m^3 tanks. Both TAN and turbidity increased during Phase 2 in the 190 m^3 → 103 m^3 tanks as the density increased, as also found by, e.g. Luz, Silva, Melillo, Santos, Rodrigues, Takata, de Alvarenga and Turra (2012). Despite the significant differences in pH, CO_2, turbidity and TAN in the 190 m^3 → 103 m^3 tanks, the growth rate in this group was still highest. This is most likely because the maximal values of the mentioned parameters (Table 1) are still below what is expected to adversely affect growth (Sweka & Hartman 2001; Fivelstad, Olsen, Åsgård, Bæverfjord, Rasmussen, Vindheim & Stefansson 2003; Kolarevic, Selset, Felip, Good, Snekvik, Takle, Ytteborg, Bæverfjord, Åsgård & Terjesen 2013; Fivelstad, Kvamme, Handeland, Fivelstad, Olsen & Hosfeld 2015).

Despite from higher mortality in 190 m^3 → 3 m^3 tanks compared to 0.9 m^3 → 3 m^3 tanks, the results from this study suggest that tank scale prior to the experiment (i.e. history) does not affect later growth and performance, as long as the grow-out period occur in tanks of equal size and otherwise are treated equal (Research question 2). Fish in all 3 m^3 tanks, irrespective of original Phase 1 scale did not differ from each other regarding condition factor, SGR, TGC and FI (190 m^3 → 3 m^3 = 3 m^3 → 3 m^3 = 0.9 m^3 → 3 m^3). In this regard, it is noteworthy that fish which were transferred to the smaller 0.9 m^3 tanks (190 m^3 → 0.9 m^3), showed significantly lower growth rate. The experimental design did not test the outcome in 0.9 m^3 tanks with different scaling history. However, the results from this study suggest that the worst scenario for fish performance in tanks is to move them across large scale differences, such as the 190 m^3 → 0.9 m^3 change. The better performance in 190 m^3 → 3 m^3 tanks suggests that 190 m^3 → 3 m^3 was a threshold for this experimental design and that 190 m^3 → 0.9 m^3 should be avoided in future experiments. In addition, a high mortality just after transfer was observed among the 190 m^3 → 0.9 m^3 fish, as discussed above. These fish showed lower feed intake, grew less and they were less active in the tanks. Behavioural

observations showed that these fish drifted backwards to a higher degree; a behaviour that results when the fish are not strong enough to withstand the water current. The inferior performance of the 190 m^3 → 0.9 m^3 fish may, however, not only be a result of the small tanks *per se* but also the magnitude of change, since the fish that were transferred from one small tank to another small tank (3 m^3 → 3 m^3 and 0.9 m^3 → 3 m^3) performed significantly better than the 190 m^3 → 0.9 m^3.

This study also aimed at comparing performance in tanks with commercial cages by transferring fish directly from the truck to 3 m^3 tanks (2 m → 2 m) and comparing these with three commercial cages (each 17 000 m^3) at Korsneset (Research question 3, Fig. 1). Accumulated mortality in cages (6.5–9%; M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F. Terjesen, submitted), exceeded the 3.5% mortality that was observed in the 3 m^3 → 3 m^3 tanks. However, some fish in the cages were infected with Pancreas disease during the experiment, and this may have contributed to the higher mortality. When comparing growth in the tank experiment with the parallel cage experiment (Espmark, Kolarevic, Åsgård, Willumsen, Lange, Alfredsen, Alver, Føre, Senneset, Birkevold & Terjesen 2014; M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F. Terjesen, submitted), the SGR (1.17 ± 0.01) and TGC (2.8 ± 0.10) in the cages were not significantly different compared to fish in the 3 m^3 → 3 m^3 tanks. However, although not part of the original design, the growth rate in the cages were significantly lower when compared with the 103 m^3 tanks ($t = 5.33$; $P = 0.003$). Although there are several factors that may limit growth, such as diseases and fluctuating conditions that can occur more often in cages, these results show that there are potential for improving salmon growth rate in the farming industry. The comparisons between these cages and tanks also show that the results that were obtained in the 3 m^3 research tanks are representative for growth rates in the much larger industrial cages. Thus, when fish performance is the target variable, the industry may rely on the results that are obtained in the 3 m^3 tanks.

The results from this study may have consequences for the interpretation of previous research. Different studies, asking similar questions, using the same species of the same size, but performing the studies in different tank or cage sizes, may obtain different results where the explanation does not take into account the unit size. The importance of including experimental design into the explanation of results was also pointed out by Adams, Turnbull, Bell, Bron and Huntingford (2007). Also, once the tank size effects is documented, it is believed that this study may have consequences on future research. It has already been mentioned that some of the present results are being used in modelling work (M. Føre, M. Alver, J-A. Alfredsen, G. Marafioti, G. Senneset, J. Birkevold, F-W. Willumsen, G. Lange, Å. Espmark & B.F. Terjesen, submitted), thus results obtained in smaller tanks may be of more industrial relevance if they are used to model performance in large tanks and cages.

Conclusions

The results presented in this study are of significance to the aquaculture industry, as well as the research community. First, the study indicates that in experiments with Atlantic salmon post-smolts, the fish exhibit a higher growth rate when reared in large vs. small tanks, as demonstrated in Research questions 1. However, as long as the growth period takes place in tanks of equal size, it does not seem to matter that earlier life stages have occurred in different sized tanks (Research question 2). This finding has importance for design of experiments, where growth rates are one of the target variables. The study also shows that sufficient acclimation time before start of an experiment is important, since this study demonstrated long acclimation time before transfer, and effects of handling stress after transfer. How long acclimation time that is needed, should be a focus for future studies. For the industry, an advice from this study is the industrial relevance of experiments performed in the 3 m^3 tanks, where the growth was comparable to the reference sea cages (Research question 3). In addition, the experiments indicate a potential for increased growth rate in cages, since the growth rate in the 103 m^3 tanks exceeded the growth in the parallel cage experiment. Finally, the tank size effects demonstrated in this study originated from dependencies between multiple factors, such as water quality, biomass, FI, physiology and behaviour that further illustrates the industrial relevance since they are direct consequences of tank size.

Acknowledgments

This study was partly funded under the EU seventh Framework Program by the AQUAEXCEL project N°262336: AQUAculture infrastructures for EXCELLence in European Fish Research. The smolts were delivered by SalMar Settefisk AS at Follasmolt, and the reference sea cage experiment was performed by ACE (AquaCulture Engineering) at SalMar farming in Korsneset.

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