Design, dimensioning, and performance of a research facility for studies on the requirements of fish in RAS environments

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A B S T R A C T

Recirculating aquaculture systems (RAS) are increasingly being used for Atlantic salmon smolt production. However, knowledge of how the RAS environment affects welfare and performance of Atlantic salmon is limited. For instance, safe limits for chronic exposure to typical compounds in RAS, such as NH3-N, NO2-N, and CO2 should be established for Atlantic salmon, as well as their interactions with nutrition, other RAS water compounds, and the microbiota. These questions can best be answered in a research facility that is providing a RAS environment. In addition, the facility described here was required to produce 480 000 smolts annually, to provide sufficient research fish in the institution. Design and dimensioning of such a facility require attention to flexibility for various experimental designs, and the flexibility to vary specific water quality constituents, properties that are not necessary in a standard production plant. A research facility of 1754 m2 ground floor area (Nofima Centre for Recirculation in Aquaculture, NCRA), was designed and constructed for these purposes at a cost of 45 mill. NOK (2010 value). The facility included six experimental halls, a number of support rooms, and four independent RASs. Water quality requirements at maximum feed loading were in the design phase set to <10 mg/L CO2, <0.7–1 mg/L TAN, and <0.1 mg/L NO2-N, and the RASs dimensioned with this objective. The facility was designed so that water from different RAS or flow-through water sources could be chosen at the level of the culture tanks, thus giving flexibility for experimentation. Performance of the facility was tested in two trials, during the first 3 years of operation. In Trial 1, a standard production study showed that Atlantic salmon parr reared in the facility had growth rates comparable to that seen in the Norwegian Atlantic salmon smolt industry. In Trial 2, water quality and removal efficiencies of RAS 1 were evaluated at increasing daily feed loads. Removal efficiencies were comparable, in the case of TAN, and when calculated for the system as a whole also for CO2, to assumptions made during dimensioning and design of the facility. The RAS maintained water quality within set limits for TAN and CO2, but not in the case of nitrite (0.22 mg/L NO2-N versus 0.1 mg/L limit). The water quality limits of TAN and CO2 were reached, not at full feed capacity, but at 134% of the theoretical feed capacity calculated prior to construction. This dimensioning was based on an often used methodology. When recalculating the RAS 1 TAN production, but now using published Atlantic salmon parr N-retention data, it was found that the methodology used prior to construction may over-estimate the TAN production by about 34%. Thus, Trial 2 was useful for recalibrating the feed load capacity of the RASs, and for accurate experimental design in future projects. It is expected that in the long-term NCRA will be useful in determining the environmental and nutritional requirements of fish reared in RAS.

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Abbreviations: BW, body weight; CO2, carbon dioxide; COD, chemical oxygen demand; DO, dissolved oxygen; DM, dry matter; FT, flow-through; GRP, glass-fiber reinforced plastic; GSM, global system for mobile communication; HDPE, high-density polyethylene; HLR, hydraulic loading rate; HMI, human machine interface; LUX, liquid oxygen; MBBR, moving bed bio-reactor; N2, nitrogen; NO2-N, nitrate nitrogen; NO3-N, nitrite nitrogen; ORP, oxidation reduction potential; PLC, programmable logic controller; PVC, polyvinyl chloride; RAS, recirculating aquaculture system; RQ, respiratory quotient; TAN, total ammonium nitrogen; TSS, total suspended solids; UV, ultraviolet; VHF, very high frequency.

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1. Introduction

Recirculating aquaculture systems (RAS) for Atlantic salmon (*Salmo salar* L.) smolt production are few in Norway, due to historically sufficient freshwater bodies for the required water use. Most salmon smolt producers in Norway use flow-through (FT) systems, or partial reuse systems with tank-level CO₂ degassing. However, Kittelsen et al. (2006) showed that further increases in smolt production in Norway beyond 2012 may be hampered without implementing water treatment that can lower the water usage, such as RAS. Future increases in smolt production in combination with water resources that are already leveraged at near maximum capacity has therefore promoted interest in RAS in Norway. To support such a development, it was decided to establish the Nofima Centre for Recirculation in Aquaculture (NCRA) at Sundalsløra, Norway (62°40′1.24″, 8°31′28.14″). A project group determined the long-term research goals to be achieved in the facility, as well as the requirements to be met by the facility. Studies describing effects of RAS on Atlantic salmon welfare or performance are relatively few (Risa and Skjervold, 1975; e.g. Sutterlin et al., 1984; Bowser et al., 1989; Eikeybrokk and Ulgenes, 1998; Griffiths and Armstrong, 2000; Rusten et al., 2006). It was therefore decided that the primary purpose of NCRA should be to facilitate studies on effects of RAS on the fish and to provide recommendations on how to achieve optimal performance, health and welfare in RAS. The project group concluded that studies on Atlantic salmon were needed in particular on subjects such as (1) safe chronic ammonia and nitrite levels in RAS, (2) effects of fish density determined in RAS environments, (3) thermal optima for low malformation rates of salmon in RAS, as have been studied in FT (Baverfjord et al., 1999), and (4) optimal tank water velocity during parr production in RAS. Although the facility was thought to be useful also for testing RAS equipment, it was decided that technological research activities should be secondary to fish requirement studies. A second objective of the facility was to produce up to 480 000 smolts annually, for use in experiments in Nofima, and in commercial-scale experiments together with the aquaculture industry.

A research facility to meet these objectives called for a different project approach compared to planning a RAS plant for commercial production purposes only. In addition, the research facility had to be accurately dimensioned, and its performance documented, such that good experimental designs can be developed in research projects. Furthermore, the growth rate of the studied fish species, at control group conditions, should be higher or at least comparable to growth rates obtained in the aquaculture industry.

One possible solution to the set of requirements described above is reported here, as well as performance data obtained during the first 3 years of operation. The project development, facility design and dimensioning, described here may be of use to other research institutions that intend to establish similar or related facilities.

2. Materials and methods

A pre-project group was established to determine the requirements that the facility should fulfill, and the overview of the design and dimensioning necessary to meet these requirements. Subsequently, a main project group was established, which supervised construction (as owner’s representatives), start-up and performance evaluation of the facility. The total cost for establishing NCRA was 45 mill. NOK, 2010 value (7.6 mill. US$, at 5.9 NOK/$).

2.1. RAS dimensioning and design

Dimensioning of the reuse systems was done according to mass-balance principles, as outlined by several authors (e.g. Liao and Mayo, 1972; Losordo and Hobbs, 2000; Summerfelt and Vinci, 2004a,b; Vinci et al., 2004; Eding et al., 2006; Timmons and Ebeling, 2007). Growth rates used in the calculations at various fish weight and temperatures were according to Club N 2002 tables for Atlantic salmon (Skretting, 2007). Dimensioning of multi-chambered moving bed bioreactors (MBBR) followed that of Drennan et al. (2006) and Rusten et al. (2006). Regarding dimensioning and design of CO₂ degassers, equations developed by Summerfelt et al. (2000) were used for calculations of packing height, flow distribution plate area, and required countercurrent air flow in the degassers.

2.2. Start-up of systems used to evaluate initial facility performance

A total of four separate RAS were installed in the facility, as described below. However, RAS 1 was used for most of the performance testing. RAS 1 MBBR was started for the first time in May 2009, by adding media to ground well water gradually due to the hydrophobicity of the media, until 10.5 m³ packed volume (sum of all three MBBR chambers) was reached at the 2nd of July, 2009. Nitrification was started by using ground well water, feed extract, and dosing of NH₄Cl (to initially 5 mg/L TAN), NaNO₂ (initially 0.5 mg/L NO₂-N), NaH₂PO₄ (0.1 mg/L PO₄-P), and NaHCO₃ (75 mg/L as CaCO₃). During the start-up phase the temperature was 20±3°C (mean±SD). No fish tanks were connected to RAS 1 until May 2010. RAS 2 and grow-out hall 1 RAS were started similarly in April 2010, except that in the case of grow-out hall 1, media was added until 16.2 m³ packed volume: i.e., the amount of MBBR media used during the first rearing was less than for full capacity (see Section 3.1.8).

2.2.1. Trial 1: Evaluation of growth rate of Atlantic salmon parr reared in NCRA

Atlantic salmon parr of SalmoBreed strain (SalmoBreed AS, Bergen, Norway), were reared until 6.5 g mean individual weight in a FT system in a separate facility at Nofima Sundalsløra. To evaluate growth rate in NCRA, the fish were stocked in June 2010 in three 3.3 m³ tanks (5115 individuals per tank) in Experimental hall 1, and received water from RAS 1. The set-up of RAS 1 during Trial 1 was as described under Section 3.1.7 below, with the one exception that ozone (O₃) supplementation was not used, since construction of the dosing system was not completed by the time Trial 1 started. The fish were fed commercial diets with 1–4 mm pellet sizes throughout the study (EWOS, Bergen, Norway). The daily ration to the tanks were adjusted so that a minimum amount of waste feed accumulated in the swirl separators connected to each tank.

2.2.2. Trial 2: Evaluation of system water quality and treatment efficiencies at maximal daily feed load

Water quality and unit treatment efficiencies were measured in Trial 2. In this trial the fish biomass and corresponding total system feed loading approached (42 kg feed/day), exceeded slightly (49 kg feed/day), or exceeded significantly (61 kg feed/day), the maximal theoretical daily feed loading capacity of RAS 1 (45 kg feed/day), determined during dimensioning prior to constructing the facility (Tables 1–3). Operating conditions were otherwise as equal as possible to the maximal load situation listed in Table 2 for RAS 1. System configuration during Trial 2 was as described under Section 3.1.7 below for RAS 1, with the one exception that flat horizontal tank outlet sieves were used in the experimental tanks, and not the Eco-trap outlets (AquaOptima, Trondheim, Norway), due to the small size of the fish at stocking. During Trial 2, O₃ was supplemented to RAS 1 using ORP set-points of 270 mV (dosing on), and 275 mV (dosing off). At Day 0, 199 000 Atlantic salmon parr of 2.8 g/ind size (Bolaks strain, Eikelandosen, Norway)
Table 1
Water quality parameters of degassed ground water at Nofima Sunndalsøra.

| Parameter         | Unit | 11 April | 3 Nov | Mean | SD  |
|-------------------|------|----------|-------|------|-----|
| Conductivity      | Cond (mS/m) | 1.6 | 45.8 | 57.2 | 25.7 |
| pH                | –    | 6.4 | 6.7 | 7.0 | 0   |
| Calcium           | Ca (mg/L) | 1.7 | 4.6 | 10.9 | 3.5 |
| Magnesium         | Mg (mg/L) | 0.2 | 8.0 | 10.1 | 5.4 |
| Sodium            | Na (mg/L) | 0.7 | 65.7 | 83.8 | 39.5 |
| Potassium         | K (mg/L) | 0.2 | 2.7 | 3.8 | 1.1 |
| Aluminum          | Al (µg/L) | 21 | 15 | 12.8 | 4.4 |
| Sulphate          | SO₄ (mg/L) | 2.8 | 20 | 31.4 | 9.3 |
| Chloride          | Cl (mg/L) | 0.9 | 118 | 141 | 72.8 |
| Alkalinity        | Alk (µmol/L) | 60 | 79 | 216 | 11.2 |
| Nitrate           | NO₃-N (µg/L) | – | – | 350 | 55.8 |
| Total nitrogen    | Tot-N (µg/L) | 78 | 90 | 456 | 28.9 |
| Total organic carbon | TOC (mg/L) | 0.6 | 0.8 | 0.2 | 0.1 |
| Iron              | Fe (µg/L) | 15 | 11 | 6.6 | 2.6 |
| Turbidity         | Turb (FNU) | 0.2 | 3 | 0.1 | 0.1 |
| Carbon dioxide    | CO₂ (mg/L) | – | – | 7.0 | 1.4 |

Water sampled at two dates in 2000, and during spring 2003 (n = 6) and analyzed by Norwegian Institute of Water Research. The 2003 time series represent unpublished data (T. Kristensen, NIVA, by permission).

Table 2
Maximum load situations, and the required water quality at these situations.

| Parameter         | Unit | RAS 1 or 2 | Grow-out hall 1 or 3 |
|-------------------|------|------------|----------------------|
| Maximum load situation |      | RAS 1 or 2 | Grow-out hall 1 or 3 |
| Temperature       | ºC   | 14         | 14                   |
| Water source      |      | Ground water | Ground water |
| Rearing volume    | m³   | 48         | 300                  |
| System hydraulic retention time | Days | 0.5–5     | 0.5–5                |
| Fish size         | g    | 2          | 50                   |
| Fish density      | kg/m³ | 25         | 50                   |
| Growth rate       | % BW/day | 42        | 2.5                  |
| FCR               | feed/gain | 0.9      | 0.8                  |
| Feed load         | kg feed/day | 45       | 303                  |

Water quality at maximum load

| pH                | 6.8–7.2 | 6.8–7.2 |
| CO₂              | <10     | <10     |
| O₂ sat           | 90–120  | 90–120  |
| TAN              | <0.7    | <1      |
| Nitrite          | mg/L NO₃-N | <0.1  | <0.1                |

*CO₂ to be varied for experimental purposes at maximum load.

were stocked in a total of nine tanks of 3.2 m³ each in Experimental hall 1, and reared under continuous light. The fish were fed a commercial feed (Skretting Nutra Olympic 1.2–1.5 mm, Skretting, Stavanger, Norway), provided evenly over 24 h. During the first period of the trial, up to Day 20 since stocking, feed loading was gradually increased to 40 kg/day, according to the predicted fish growth. During the following 19 days period, feeding continued to be increased according to, or slightly below (~5%), the expected fish growth (Skretting, 2007, and using a FCR of 0.9 (Austreng et al., 1987). Conditions during Trial 2 were maintained as close to the theoretical maximal load situation as possible (Tables 2 and 3), in terms of pH, flow rate, make-up water flow rate, and temperature. At Day 21 after the initial stocking, at Day 27, and at Day 39, corresponding to the daily feed loadings of 42, 49, and 61 kg, respectively, several water samples at various RAS 1 locations were collected to determine water quality, and unit treatment efficiencies of the mechanical filter, MBBR and CO₂ degasser. The CO₂ removal efficiency measured here is reported as the apparent removal efficiency, as discussed by Colt et al. (2012).

2.2.3. Data logging and analyses

During both Trials 1 and 2, several parameters were measured using online instruments; pH (Sensorex S8000CD-pH,78, Sensorex, Golden Grove, USA) and temperature (PT100, Hypech, Drammen, Norway) were measured continuously in the degasser sump, ORP in the mechanical filter inlet (Sensorex S8000CD-ORP, in duplicate), and recycled and make-up water flow rates (Siemens Sitrans FM Magflo flow transmitters, Berlin, Germany), as well as O₂ saturation in each experimental tank (Sensorex DO6442-T). These data were logged every 5 min.

Water quality during Trial 1 was measured regularly using a Hanna Instruments C203 2008 photometer (Hanna Instruments, Quebec, Canada) with reagents for total ammonia nitrogen (TAN, sum of NH₃-N and NH₄-N) determination (method HI 93700) and for nitrite nitrogen (NO₂⁻N, method HI 93707).

Water samples collected during Trial 2 were obtained by siphoning from sumps or the biofilter outlet, or from sampling outlets on pipes (e.g. tank inlets). In Trial 2, TAN, NO₃-N and NO₂⁻N concentrations in batch water samples were analyzed using an automated analyzer (Flow Solution IV, O1 Analytical, College Station, TX, USA), according to U.S. E.P.A Method 350.1 (U.S.EPA, 1983) for TAN and U.S. E.P.A Method 353.2 (U.S.EPA, 1983) for NO₃-N and NO₂⁻N. Total inorganic carbon (TIC) was analyzed on fresh samples kept on ice, collected without air-bubbles in glass flasks, according to method 6/93 Rev. B (Perstorp Analytical, Perstorp, Sweden). In this analysis, the sample was acidified in the FSIV autoanalyzer, and the resulting CO₂ diffused through a semi-permeable membrane, which lowered pH and lead to color loss in a weakly buffered phenolphthalein solution. The TIC concentration was calculated from sample readings and TIC standard curves using WinFlow software (Ver. 4.2, O.I. Analytical). CO₂ in the original sample was subsequently calculated from pH and temperature measured at the time of collection, and using carbonate system constants in Summerfelt et al. (2001). CO₂ was in some cases also estimated using an Oxyguard portable CO₂ analyzer (Oxyguard, Birkered, Denmark). In addition to the online probes in the degasser sump, pH was also measured at other locations in the RAS and tanks during Trial 2, using two Hach HQ40D pH meters with Hach PHC101011 electrodes (Hach Lange, Düsseldorf, Germany), whereas conductivity was measured using a Hach CDC401 probe connected to the HQ40D meter. Water samples were analyzed for TSS according to standardized method 2540 D (TSS dried at 103–105 ºC) [APHA, 2005]. A commercial kit from WTW (Cat.# 250303) and a PhotoLab 6000 VIS series spectrophotometer (WTW, Weilheim, Germany) were used for analysis of total chemical oxygen demand (COD). Turbidity was measured on fresh water samples using a Turbidiquant 1500 IR (Merck, Darmstadt, Germany), while alkalinity was measured according to APHA [1999]. Nitrogen gas saturation of water samples at various locations in RAS 1 was
Table 3

| Parameter | Value | Unit | Reference/comment |
|-----------|-------|------|-------------------|
| Total tank volume | 48 | m³ | |
| Total biomass | 1200 | kg | |
| Total feed per day | 45.4 | kg | |
| Maximum system HRT | 5 | Days | |
| Bioreactor | | | |
| Specific protected media area | 900 | m²/m³ | |
| TAN production per day | 2083 | g | |
| TAN conc. inlet reactor CH1 | 0.70 | mg/L | |
| TAN conc. outlet reactor CH1 | 0.35 | mg/L | |
| Specific nitrification rate CH1 | 0.361 | g TAN/m²/d | |
| Needed TAN removal CH1 | 1133 | g/day | |
| Total media area needed CH1 | 3140 | m² | |
| TAN conc. inlet reactor CH2 | 0.35 | mg/L | |
| TAN conc. outlet reactor CH2 | 0.15 | mg/L | |
| Specific nitrification rate CH2 | 0.203 | g TAN/m²/d | |
| Needed TAN removal CH2 | 636 | g/day | |
| Total media area needed CH2 | 3140 | m² | |
| TAN conc. inlet reactor CH3 | 0.15 | mg/L | |
| TAN conc. outlet reactor CH3 | 0.06 | mg/L | |
| Specific nitrification rate CH3 | 0.100 | g TAN/m²/d | |
| Needed TAN removal CH3 | 314 | g/day | |
| Total media area needed CH3 | 3140 | m² | |
| Total needed media volume | 10.5 | m³ | |
| Filling factor reactor | 50 | (%) media | Suppliers statement |
| Total reactor volume (CH1 + 2 + 3) | 21 | m³ | |
| Required TAN removal efficiency | 92 | % | Single pass |
| Degasser | | | |
| Oxygen demand/kg feed | 350 | g O₂/kg feed | Assuming an RQ of 0.85, Kieffer et al. (1998) |
| Carbon dioxide prod/kg feed | 409 | g CO₂/kg feed | |
| Total CO₂ prod. per day | 19 | kg | |
| CO₂ conc. inlet degasser | 10 | mg/L | |
| Hydraulic loading rate | 0.025 | m³/s/m² | |
| Required CO₂ removal efficiency | 78 | % | Single pass |
| CO₂ conc. outlet degasser | 2 | mg/L | |
| Packing height minimum, Z | 1.6 | m | |
| Packing area | 1.5 | m² | |
| Gas:liquid ratio, countercurrent | 5 | | |
| System water flow | | | |
| Flow req TAN removal | 2.2 | m³/min | |
| Flow req CO₂ removal | 1.7 | m³/min | |
| Tank HRT at control flow | 21 | min | |

calculated from total gas pressure measurements using a P4 Tracker and/or a TBO-F (Pointfour, Coquitlam, BC, Canada). All handheld instruments were calibrated according to manufacturer’s instructions. In the case of online pH probes, two-point calibrations were done each week, while the OxygenGuard CO₂ probe, and the handheld pH meters were calibrated before each sampling point. In the case of the pH, the meters were also cross-checked against each other.

Subsamples of the feed used during Trial 2 were collected at each water quality sampling point or when changing feed pellet size, and analyzed for dry matter (DM, 105 °C until constant weight), crude lipid after HCl hydrolysis (2055 Soxtec Avanti and SoxCap system 2047 Hydrolyzing Unit, FOSS, Hillerød, Denmark), crude protein (N x 6.25; Kjeltec Auto 2300, FOSS), ash (550 °C overnight), and energy (Parr 6300 bomb calorimeter, Parr Instrument Company, Moline, IL, USA).

3. Results and discussion

3.1. Dimensioning and design

3.1.1. Required capacities in NCRA

The pre-project established that the facility should cover two requirements: experimental objectives and experimental fish production goals. Although the facility was designed to cover two objectives, it was not required that both requirements were met at full capacity, at the same time. The experimental objectives were prioritized over the production goals. The experimental design of two planned studies (“Experiment 1” and “Experiment 2”) were used to determine the number of experimental treatments necessary to provide in the facility, since these trials were deemed to be the most complex and resource intensive trials that would be done in the foreseeable future. The first experiment, called temperature tolerance of salmon parr in RAS versus FT, was found to require 12 tanks, two RAS and two FT water sources, over two water temperatures, in a two-way design (water source × temperature). The second experiment, named long-term effects of RAS versus FT during Atlantic salmon smolt production, was planned to occur from about 5 g/ind size, through smoltification and sea transfer, until ½ year at sea in cages at Nofima’s station at Averøy (63°3′38.43″, 7°35′28.65″). In NCRA, this experiment required systems for maintaining similar temperatures in RAS and in FT tanks, and a minimum of eight tanks of sufficient volume to produce 500 smolts per tank, for stocking in experimental sea cages (125 m³ volume each cage). The second requirement concerned production capacity for experimental fish. Nofima requires fish of several sizes during the year for small-scale experiments and to enable large-scale trials in collaboration with the industry. The facility was required to produce up to 480 000 Atlantic salmon smolts annually, ready for sea
3.1.3. Overview of chosen solutions

The main outcome of the pre-project was to build a two-storey building of 1754 m² (ground floor) and 553 m² (second floor) area (Figs. 1 and 2). At the ground floor, three small-scale research halls (with a total of 48, 0.5–3.2 m³ tanks) were built, served by two separate RASs (RAS 1 and 2). A central water treatment room was constructed to house water intake, water pre-treatment (mixing, heating, degassing), and RAS 1 and 2 equipment. Further, three grow-out halls were built (three, 100 m³ tanks in each hall). Two of these halls were constructed with one RAS in each (grow-out halls 1 and 3), while one hall was built with FT only (grow-out hall 2). The entry rooms to the halls, and the central water treatment room, were designed for biosecurity control with a central clean zone.

At the second floor a number of support components were included: control room (47 m²), power distribution and control systems (37 m²), rooms for ventilation systems and MBBR blowers (100 m²), a wet-area for logistics (sorting and vaccination), including feed distribution to the grow-out halls (156 m²), a laboratory for fish sampling and basic water quality analysis (42 m²), meeting room (39 m²), and viewing corridors (93 m²) with windows towards grow-out halls and central water treatment.

Water pipes installed in the facility were of HDPE material, with some use of PVC at the low-pressure side from Experimental halls 1–3. All low-pressure pipes were dimensioned to maintain a minimum water velocity of 0.6 m/s, to avoid sedimentation of biosolids. To give experimental flexibility in Experimental halls 1–3, a total of four water sources were installed to supply each tank (RAS 1, RAS 2, and two FT sources).

To control the water treatment systems in the facility and log data, two programmable logic controllers (PLCs; NSJ8, Omron, Kyoto, Japan) were installed together with electrical components and wiring in 10 cabinets, in a temperature-controlled room (−15 °C) at the 2nd floor. The speed of all water pumps and mechanical filter belts, except the booster pumps ahead of the O₃ injectors, were controlled by variable frequency drives (Varispeed series, Omron), which received input from pressure or level transmitters, via the PLCs. For control, monitoring and logging purposes, a human machine interface (HMI) system was built (CX-Supervisor ver. 2.2, Omron), displayed on four monitors, and data logged usually each fifth minute. The system was made accessible externally via virtual private network and remote desktop software. Alarm-systems were included at two levels. Firstly, the PLCs were programmed to send unspecific alarm-signals to the duty guard via a very high frequency (VHF) radio system. Secondly, specific alarms (e.g. “low DO in Tank 8 Exp. Hall 1”), were programmed to be sent from the HMI using the global system for mobile communication (GSM).

3.1.4. Scaling and number of replicate RAS

Regarding the choice of the relatively large experimental fish tanks and RASs, it has been shown previously that Atlantic salmon grow faster in large versus small tanks (Bœuf and Gaïgnon, 1989). Studies on the effects of tank and RAS biofilter scale (e.g. volume), on salmon growth and biofilter kinetics, respectively, are ongoing in a EU-FP7 Infrastructures project (Vandeputte and Reuver, 2011), in which NCRA participates. Since growth rate influences a number of physiological mechanisms and nutritional requirements in fish (e.g. Wood, 2001) a relatively large scale was chosen for experimental tanks and RASs in the facility, to ensure that relevant growth rates were obtained. Regarding the number of independent RAS in the facility (four), this was chosen as a consequence of the main requirement, to study the physiological requirements of fish in a RAS environment. Hence, the main study object was the fish, at tank level, and the effects of changing water quality. In this type of research, in contrast to technology studies, it was reasoned that a single RAS, with treatments replicated at the tank level, provides a more proper experimental design than using several replicate RAS. Using a putative ammonia tolerance study in RAS as an example, a single RAS will be able to provide the same basal water quality to all experimental tanks and treatments, unless if several replicate RAS are used, due to RAS- to-RAS variation. The ammonia experimental treatments can be set-up by dosing on the water inlet pipe or to holding tanks just prior to each experimental tank, to reach the intended treatment concentration. The TAN load in the return line can be removed in the biofilter, to generate control level TAN concentration again in the MBBR outlet, by using a properly dimensioned and calibrated MBBR. In this way, a similar water quality, except the TAN level, can be obtained in all the experimental treatments, unlike what will be the case if several replicate RAS were to be used in the study.

3.1.5. Intake water sources

In the central water treatment room four main intake pipes were installed (Figs. 1, 2 and 4B) named Sea water, Fresh 1 and 2, and Backup (Fig. 4B), originating from the freshwater and sea water central intake treatment facilities at Nofima Sundalsløret, external to NCRA. All sea water entering Nofima Sundalsløret is filtered to 10 μm in disc microscreens (Hydrotech, Vellinge, Sweden), and UV-irradiated at 45 mJ/cm² (Berson InLine 750, Berson Milieutechniek, Nuenen, The Netherlands). Annual temperature in the ground freshwater (from three separate wells, 18–20 m bore depth) was found to vary between 6 and 9 °C, while the sea water varied more...
Fig. 1. 3D sketch of the NCRA facility. A, overview, without second floor and roof; B, viewed towards the grow-out halls; C, viewed towards the central water treatment hall and experimental halls. Only the corridor section of the 2nd floor is shown. Drawing by Nofima, with components from AquaOptima (Trondheim, Norway) and 3S Prosjekt (Molde, Norway).
Fig. 2. 3D-sketch of NCRA, showing the main water treatment systems, tanks and piping. The actual constructed facility differs in minor aspects, such as oxygenation cones and tank inlets being located behind the tanks in the grow-out halls, and that grow-out hall 1 also has side wall drains. Several details of the building and water treatment have been omitted for clarity, such as the entire ground floor concrete and 2nd floor (except room floor for MBBR blowers), walls, concrete structures, ventilation, and piping for grow-out hall 2. All of RAS 1 and 2, the pump sumps of grow-out halls 1 and 3, and the large holding tank, are situated at ~1 m relative to ground floor level. Drawing developed by Nofima, AquaOptima (Trondheim, Norway) and 3S Prosjekt (Molde, Norway).

Fig. 3. Process flow drawing of the RAS in the facility, exemplified with RAS 2 (components are not drawn to scale). All other RAS in the facility follow the same concept. Only three fish tanks are shown, out of the 48 tanks that can receive RAS 2 water. The moving bed bioreactor contains three chambers, and flow to each can be regulated, but flow was typically only added at the head of the farthest chamber. Refer to Section 3.1.7 for description of components marked by numbers 1–12.

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The ground well freshwater (Table 1) was found to have relatively high ionic strength and pH, when compared to other sites in Norway (Kristensen et al., 2009). However, water chemistry in the ground water wells varied during the year, in particular conductivity, typical marine sediment ions, and pH (Table 1). One of the three ground water wells was more influenced by marine sediments than others (data not shown), which likely contributed to this variance.

Although all water sources are degassed at Nofima Sunndalsøra, prior to entering the NCRA facility, a second degassing step (cascade columns) were installed in the central water treatment room, to improve security against gas super saturation. Since the ground well water can be relatively high in CO₂ (Table 1, 7.0 ± 1.4 mg/L), the additional degassing step was also installed to decrease CO₂ levels of make-up and FT water. Circular polyethylene degassing columns of 2 m height, 0.63 m diameter were installed, with random packing of Nor-Pac rings (5 cm diam., Jaeger Environmental Products, Houston, TX, U.S.); however, these degassers did not include spray nozzles or countercurrent air blowing. CO₂ in the degasser effluent water was monitored with the Oxyguard analyzer in one of the holding tanks between June and August 2011; the CO₂ concentration averaged 2 mg/L at a flow of 200–300 L/min, temperature of 13.5 ± 1.2 °C, and pH of 7.1 ± 0.2.

A fifth intake pipe, supplying hot waste-water was installed by a local company (Sunndal Energi, Sunndalsøra, Norway) to distribute water containing excess heat generated at a nearby aluminum plant (delivered at 85 ± 3 °C according to the supplier). This waste-water was utilized at three locations in NCRA; (1) in a heat-exchange system in the central water treatment room, for heating FT or make-up water (sea or freshwater), (2) in the ventilation room at the 2nd floor for temperature control of the intake air to RAS CO₂ degassers (to 15 °C), and (3) for heating air in the standard ventilation systems. For use in FT or RAS, the water was pumped through a two-step titanium heat exchanger system (GEA Ecolflex, Sarstedt, Germany), situated in the upper right corner of the central water treatment room (indicated by a grey box in Fig. 2). The waste-heat water was passed through the first exchanger only, which carried the energy at a temperature of 28 °C in a closed circuit to a second, larger exchanger, which exchanged heat with the FT or RAS make-up water intended for the fish (6–9 °C inlet, target outlet temperature 12 °C). The required temperature was maintained by a PLC (Siemens, Munich, Germany), controlling two motor-values that regulated the flow past the last exchanger. The heat-exchanged water finally entered a degasser (2 m height), to limit gas super-saturation of the water. Performance of this waste-heat system was evaluated over two periods. Firstly, the system was set to control the outlet to 13 °C, and monitored for a week, with recordings each 5 min. Secondly, in an experiment using FT or RAS water for Atlantic salmon parr (“Experiment 2” above), the temperature of the FT water (150–450 L/min) was set to the prevailing RAS 1 temperature and the relative difference between the systems was evaluated over 16 weeks. In the first test, the waste-heat system maintained 12.9 ± 0.1 °C (n=882) in the sump below the degasser, subsequent to the heat-exchange system. In the second test, the FT heat-exchange system showed a mean temperature of 13.9 ± 0.7 °C (n=35 733), close to that of RAS 1 (13.8 ± 0.8 °C, n=35 735), and deviated from RAS 1 by 2.4 ± 2.4% (treated as positive numbers, irrespective of direction of deviation). Maximum deviation was 63%, due to a water level alarm in a RAS 1 pump sump that necessitated the use of cold make-up water for some hours, before personnel was able to adjust the FT temperature. In conclusion, the heat-exchange system is adequate for providing water with stable temperature to experiments, as long as the required temperature is above that of the inlet water.

The backup water source was designed to provide either river surface water (subject to biosecurity restrictions), ground well water, sea water, or turbine/cooling water from a nearby hydropower plant. When used for emergency purposes the backup water source was installed directly to all nine 100 m³ tanks in the grow-out halls (pipes not shown in Fig. 2), bypassing pumps and RASs, thereby improving safety in case of equipment failure or if the emergency power generator (1250 kW) should malfunction. In contrast, the sea water, fresh 1 and fresh 2 intake sources, were supplied as RAS make-up water or directly as FT to the experimental tanks, following degassing, pumping and oxygenation. The turbine/cooling water source was installed due to its low temperature (<4 °C for several month annually), to provide cooling for the CO₂ degasser air temperature control system and for the other ventilation systems at the 2nd floor.

To be able to use sea water, and to prolong pump service life, all pumps were dismantled prior to installation and the components in direct contact with water treated with a ceramic coating (Chesterton, Woburn, MA, USA).

3.1.6. FT systems

All tanks in Experimental halls 1–3 were built for RAS or FT mode, and designed so that either of four water sources could be chosen at random (pipe selection shown in Fig. 5B). The required FT water flow rate to halls 1–3 was estimated according to Experiment 1 (0.9 m³/min), and a centrifugal pump (nominal 2 m³/min, 12 m head, 5.5 kW, ITT Flygt, Sundbyberg, Sweden) was thus installed at each of the smaller holding tanks. Grow-out hall 2, however, was configured as a FT system only, for comparisons with RAS at a larger scale. Other than the lack of RAS in grow-out hall 2, the design of the equipment was similar to that of grow-out halls 1

Fig. 4. Leftmost panel (A), temperatures in raw sea water or ground well water. The panel to the right (B) shows intake pipes (315–400 mm HDPE) and water sources installed, prior to construction of the NCRA facility.
culture tank volume approximately once every hour. Two centrifugal pumps (ITT Flygt) were installed for this purpose, each rated to a nominal 4 m³/min at 13 m head (16 kW).

3.1.7. RAS design overview

The reuse systems were installed in the central water treatment room (RAS 1 and 2), and in the grow-out halls 1 and 3 (Figs. 1–3). The purpose of RAS 1 or 2 were experiments on smaller sized fish (0–200 g BW) and production of experimental fish to transfer to grow-out halls at ~7 g BW. In the central water treatment hall, RAS 1 and 2 equipment was installed at ~1 m relative to ground level. In the grow-out halls however, only the microscreen and the pump sump after the degasser, was situated at ~1 m.

Each of the RASs in the facility was built with the following main equipment parts, in the flow direction starting from the tanks (refer to numbers in circles in Fig. 3): (1) octagonal experimental fish tanks in glass-fiber reinforced plastic (GRP; Namdalsplast, Namdalseid, Norway) in the experimental halls or aluminum in the grow-out halls (AquaOptima, Trondheim, Norway), (2) particle trap in the tank center and swirl separators outside the tanks (AquaOptima Eco-trap, e.g. Losordo et al., 2000), (3) side wall drain (AquaOptima) as applied in Cornell-type dual-drain tanks (Davidson and Summerfelt, 2004), (4) O₃ supplementation on a side-stream, added to the return line before the belt filter (Ozonnia CFS-14 2G generator, Degremont Technologies, Dübendorf, Switzerland; Mazzei GDT 60 g or 300 g O₃ injectors, degas separators, and O₃-destruct units, Bakersfield, CA, US, and online Sensores 8000CD ORP probes), (5) microscreen belt filter (Salsnes SKF 400 in RAS 1 and 2 and SKF 600 in grow-out halls, Salsnes, Norway) with infastrad position control (Vegason 61, Vega, West Sussex, U.K.), (6) pre-biofilter pump sump fabricated in GRP (AquaOptima) with three 1.5–7.5 kW centrifugal pumps (ITT Flygt, nominal 0.75–4 m³/min each, at 6 m head, level controlled speed), (7) three-chambered moving bed bioreactor (Kalndes MBBR with Biofilm Chip P, 900 m²/m³ area, KrügerKaldnes, Sandefjord, Norway), (8) forced-ventilated cascade aeration column in GRP (AquaOptima) with countercurrent temperature-controlled air supply, (9) alkalinity dosing system, dosing into the microscreen pump sump (Iwaki EHE or EW, Tokyo, Japan), and with online pH electrodes in degasser pump sump (Sensores 8000CD with solution ground and amplifier), controlled by a Walchem WDP 320 (Holliston, MA, U.S.). Furthermore, RAS 1 and 2 pH probes were equipped with automatic cleaning systems; a water jet programmed to flush the probe every tenth min (Storvik, Sunndalsøra, Norway, described in Kolarević et al. (2011)), (10) post-biofilter pump sump fabricated in GRP (AquaOptima), with three 3–15 kW centrifugal pumps (ITT Flygt, nominal 0.75–4 m³/min each, at 12–13 m head, pressure controlled speed), (11) online flow-meters on make-up and reuse flow (for RAS 1, 2, Sitrans FM Magflo, Siemens, Munich, Germany), (12) oxygenation system with downstream bubble contactors (AquaOptima) and DO-controller with online probes (DO6441, Sensores), at the return line close to the culture tanks: one contactor and one control circuit were used for each culture tank.

In the experimental halls 1–3, clean-outs were installed from each of the four inlet pipes above the tanks, to enable flushing of little-used pipes (Fig. 5B). The water exited the experimental halls 1, 2 and grow-out hall tanks at three locations (partly shown in Fig. 5): center drain, center particle drain, and side wall drain, the latter dimensioned to handle up to 50% of the total tank flow. On the outlet side, four pipes were installed in the floor below each tank, with a quick-lock mechanism to connect the hose from the tank to the chosen return line (Fig. 5C). These four return pipes were designated to either RAS 1 or 2 from each tank was built because treatments for the RAS tanks had to be assigned at random,
water velocity within pipes still had to be kept between 0.6 and 1.5 m/s, even if one, two or three tanks at a tank row was random assigned to return to the same RAS.

Oxygen was supplied by an external liquid oxygen (LOX) tank (6 m³), and a distribution net built into the facility to each hall, as well as the O₂-generator. In each fish tank, up to four plate oxygen diffusers were installed (Idema Aquac. Sagvåg, Norway) for emergency purposes, and the PLCs programmed to open at <70% O₂-saturation. In addition, the system was programmed to open for 1 min, four times a day, to reduce clogging of the diffuser plates.

Except the tank bottom and pump jumps in grow-out halls 1 and 3, no components of the RASs required concrete structures for installation. Instead, process units were built as stand-alone units, to make it possible to replace or modify the equipment at a later stage.

### 3.1.8. Dimensioning of TAN and CO₂ removal

Although the facility was made ready for sea water, by e.g. use of corrosion resistant materials, the dimensioning of TAN and CO₂ removal (Table 3, RAS 1) was only valid for freshwater. Thus, a decreased nitrification rate (Chen et al., 2006) and CO₂ removal efficiency (Moran, 2010) is expected during experiments with sea water. RAS 1 and 2 were designed such that both systems could supply water to each experimental tank, irrespective of whether it was in Exp. Halls 1, 2, or 3. However, each individual RAS was required to supply 15 tanks in only Exp. Hall 1 or in only Exp. Hall 2, at the same time. Exp. Hall 3 was designed for low biomass experiments and had only minor impact on dimensioning. For dimensioning of TAN removal in RAS 1, a production of 46 g TAN/kg fish was assumed, calculated from a feed of ~50% crude protein, assuming digestibility and excreted nitrogen as outlined by Timmons and Ebeling (2007). The outlet TAN concentration from each MBBR chamber was used for calculating the nitrification rate in the chamber (Drennan et al., 2006), rather than the inlet or an average TAN. A total carrier media volume of 10.5 m³ was therefore installed for RAS 1 or 2, to remove a daily TAN production of 2083 g, and maintain a maximum of 0.7 mg/L TAN in the fish tanks. This media volume was divided equally (3.5 m³ each) over the MBBR chambers, and suspended at a 50% filling factor, giving a total MBBR volume of 21 m³ (divided into three chambers of 7 m³ each). Thus, the entire MBBR was designed with a mean TAN removal rate of 0.22 g TAN per day per square meter of media. The resulting MBBR outlet TAN concentration was assumed to be 0.06 mg/L, which then entered the fish tanks again, resulting in a required single pass TAN removal efficiency of 92%. Required system flow rate to maintain 0.7 mg/L TAN in the tanks, at maximum load, was calculated to be 2.2 m³/min, giving a MBBR hydraulic retention time (HRT) of 5 min at full flow.

For dimensioning of the CO₂ degasser, a total daily production of 19 kg CO₂ for RAS 1 was estimated for the maximum load situation (Tables 2 and 3). In this calculation an RQ (MCO₂/MO₂, respiratory quotient) of 0.85 was used (Kieffer et al., 1998). This RQ may vary as the contribution of the different nutrient classes to energy dissipation in the fish varies. The MBBR may also produce CO₂ (Summerfelt and Sharrer, 2004) or strip CO₂ (depending on aeration levels), and influence the RQ when calculated for the whole system, but this was not accounted for. In the dimensioning of the CO₂ degasser, a random packing of 5 cm diameter media (Nor-Pac) was used, and parameters as in Table 2 (Summerfelt et al., 2000). The required dimensions for the CO₂ stripper were found to be 1.6 m packing height, over 1.5 m² plan area, and a hydraulic loading rate (HLR) of 0.025 m³/s/m². In the distribution plate, XF Crown nozzles (LS Entreprises, Fort Myers, FL, USA) were installed at a density of 31 nozzles/m². The degasser was assumed to keep the CO₂ concentration in the degasser effluent below 2.2 mg/L, to maintain less than 10 mg/L CO₂ in the fish tanks, i.e. a single pass removal efficiency of 78%. Such a high efficiency required, in the calculations, a minimum of five volumes of air to be passed countercurrent to each volume of water. Although tested at 20 mg/L CO₂ inlet concentration, comparable removal efficiencies have been modeled previously (Summerfelt et al., 2000). Despite showing higher removal efficiencies, random packing in CO₂ strippers give a higher risk of clogging than structured packing (Summerfelt et al., 2003). To reduce the risk of clogging, a conservative HLR was used in the facility (0.025–0.035 m³/s/m²), and the degassers were designed with three layers of packing, and access in front for replacement or cleaning.

Dimensioning of the RASs in grow-out halls 1 and 3 followed similar principles. A lower TAN load per kg feed was assumed (40 g TAN/kg feed, ~43% crude protein), since optimum dietary protein/energy ratios are inversely related to body weight in Atlantic salmon (Einen and Roem, 1997), and larger fish was intended for grow-out halls 1 and 3 than in RAS 1 and 2. A maximum feeding rate of 303 kg/day/system was used in the calculations, and 300 m³ culture tank volume per system (for either grow-out hall 1 or 3). A higher tank TAN concentration (1.0 mg/L) at maximum load was allowed for the grow-out halls. A three-chambered MBBR was installed in both grow-out halls 1 and 3, each with a media volume of 9.5 m³ per chamber, 28.4 m³ in total, resulting in a water-filled reactor of 57 m³. Thus, these two MBBRs were designed to remove on average 0.47 g TAN per day per square meter of media. The outlet TAN concentration from the reactor was calculated to be around 0.33 mg/L, indicating a required single pass removal efficiency of 67%. A system flow rate of 12.4 m³/min was calculated to be needed to maintain 1 mg/L TAN in the tanks. Regarding CO₂, it was calculated that a daily amount of 124 kg CO₂ had to be removed from each grow-out hall 1 or 3. This was found to necessitate a cascade column of 1.5 m packing height (random packing), over a 7.4 m² distribution plate area (0.027 m³/s/m² loading rate), and a 5.1 air-water forced ventilation. Required unit process water flow for CO₂ removal was 12.1 m³/min, and this dimensioning required a 75% CO₂ removal efficiency. In the water distribution plate, nozzles (XF Crown, LS Enterprises) were installed at a density of 32 nozzles/m².

### 3.1.9. Dimensioning and design of other components in the facility

Three 2.2 kW blowers (Ventrus MSB-2-355/102-220T, Ventrus Tekniska, Gothenburg, Sweden) were installed at the 2nd floor for forced air ventilation of the CO₂ degassers. The blowers were placed subsequent to the air-intake and temperature control exchanger, dimensioned to bring the air to 15 °C, from an annual range of −15 to +25 °C. Air-ducts lead air to all four CO₂ degassers in the facility. The blowers had a rated total capacity of 335 m³/min, against a 19 cm back-pressure, i.e. sufficient for running all RAS at full water flow at a G/L (gas: liquid) ratio of 10. To further ensure efficient air–water exchange, two suction blowers with a capacity of 25 m³/min at 3 cm back-pressure (Systemair P25/4, 0.37 kW, Systemair, Skinnskatteberg, Sweden), were installed in the roof above RAS 1 and 2, and another two suction blowers of 80 m³/min at 2 cm back-pressure (Systemair P30/4, 1.5 kW) were installed in the roof above each of the grow-out hall CO₂ degassers, and the air vented out of the facility.

For mixing the MBBRs with air-entry at 3.5 m depth, two side-channel blowers were installed (Busch Samos SB1100D2, 14 kW, Busch, Maulburg, Germany), each with capacity of 12 m³/min, sufficient to supply the required air for RAS 1 and 2 MBBR (total of 4.5 m³/min at 3 m depth) and grow-out halls 1 and 3 MBBRs (total of 14.3 m³/min at 3.5 m depth), running at the same time. The air-entry at depth in the MBBRs generated N₂-gas supersaturation. Measured in the microscreen inlet of RAS 2, i.e. prior to the MBBR, N₂-gas saturation averaged 95%; however, when exiting the MBBR, N₂-saturation had increased to 105%. After passing the
forced-ventilated cascade aeration column however, N₂-gas saturation was brought back to an average of 101%.

One O₃ generator was installed to supply gas to four venturi injector skids, one skid per RAS. Each skid contained a venturi injector, flash mixer, degas separator, and an O₃ destruct unit. The flow of the ozonated oxygen gas mixture to the skid was regulated by magnetic valves (Asco SCE72A049NVS3, ASCO Numatics, Paris, France), receiving open/close signals from the ORP probes, via the central PLCs. The ORP set-point was made adjustable in the HMI, and was usually set to 270 mV (open) and 275 mV (closed). Each skid was installed subsequent to either a 2.2 kW booster pump (ITT Flygt) of nominal 0.23 m³/min at heads of 33 m (RAS 1, 2), or 53 m (grow-out halls), on a 2–10% side-stream with entry before the microscreens (Fig. 3). The O₃ generator had a capacity of 17.5 kg O₃/day. Given a peak feeding rate of 697 kg/day for the whole facility (excluding grow-out hall 2), this generator was sufficient to maintain 0.025 kg O₃/kg feed as reported by Summerfelt et al. (1997) to reduce TSS, nitrite, and color in RAS for rainbow trout.

3.2. Facility performance

3.2.1. Trial 1: Evaluation of growth rate of Atlantic salmon parr reared in NCRA (Trial 1)

The fish density when Trial 1 started in Experimental hall 1/RAS 1, was 10 kg/m³. As rearing progressed, fish density increased to 22 kg/m³, and the cohort was split into another three 3.2 m³ tanks (i.e. a total of six tanks) at day 30 after initial stocking. Growth during the initial 3 weeks post stocking (2.4%/day) lagged behind weight predictions made from published growth rate tables (Austreng et al., 1987, 2.8%/day) or more recent growth rate estimates stated by feed manufacturers, based on a report system from the salmon farming industry (Skretting, 2007, 3.4%/day) (grey shaded area in Fig. 6). However, after the split into more tanks at Day 30, growth increased (2.8%/day) and became comparable to the individual weight predictions based on Austreng et al., 1987, 2.7%/day) or Skretting (2007, 2.9%/day) (Fig. 6). This situation persisted until the 2nd vaccination at Day 97, when the measured individual weights became less than predicted. However, it should be noted that the growth rates found by Austreng et al. (1987), do not account for effects due to vaccination. In addition to the described fish cohort, other groups of Atlantic salmon parr were also reared in RAS 1-connected tanks at this time. Thus, the total feed loading on RAS 1 increased from 5 to 20 kg feed/day during the studied period in this system (Fig. 7). During Trial 1 in RAS 1, the average recycled flow rate was 968 ± 334 L/min, and make-up water 30 ± 12 L/min, giving a recirculation rate of 96.9 ± 0.6% based on flow, and a daily water volume exchange rate of 81.1 ± 4.6%. At day 58 (at 51 kg/m³ density) the fish were transferred from RAS 1 and Experimental hall 1 to the grow-out hall 1 RAS, and stocked into a 100 m³ tank (initial density 5 kg/m³). During this transfer period, feed ration was decreased, based on observations of feed spill in the swirl separators, and then slowly increased. A similar drop in daily feed ration was done at Day 97 and at Day 129, during vaccination and when a second cohort was stocked into grow-out hall 1, respectively. The maximal feed loading on grow-out hall 1 RAS during Trial 1 was 33 kg/day. Hence, the load on the system was only a fraction of dimensioned capacity, both in terms of feed capacity and water exchange rate. However, due to the e.g. vaccinations, the feed loading was quite variable during the trial, which can present a challenge in RAS to maintain the required water quality (Emparanza, 2009). Ammonia and nitrite levels therefore varied (Fig. 7), especially in the period between Day 50 and 77 for NO₂-N. This period was associated with the transfer of the fish into grow-out hall 1 RAS, and the MBBR may not have been fully prepared for this increased biomass, despite the slowly increased feed loading, resulting in the higher NO₂-N. During other periods of Trial 1 however, the TAN and NO₂-N levels were well below required levels set during dimensioning. The fish were reared in grow-out hall 1 until transfer to sea cages at day 135 (final density 18 kg/m³). In conclusion, this trial demonstrated that Atlantic salmon parr could attain maximal growth rates in the facility, a prerequisite for providing industry-relevant data during experiments.

The HMI SCADA systems proved useful during start-up of the facility and in Trial 1. Firstly, malfunctions in the facility could be rapidly localized and rectified since system-specific alarm texts were sent via GSM to the duty guard, which is not feasible.
without a HMI SCADA. This feature will also be useful for commercial production facilities. The data logging feature provided by the HMI SCADA however, although useful also in commercial operations, proved particular advantageous in a research facility like NCRA, since experimental data such as flow rate, temperature and O₂-saturation could be collected at high resolution during the trial and with a low failure rate.

3.2.2. Evaluation of system water quality and treatment efficiencies at maximal load (Trial 2)

During the 3-week long build-up of feed loading, prior to the sampling for water quality started, RAS 1 water temperature was maintained at 11.2 ± 0.7 °C, and pH at 7.27 ± 0.03 (data from online probes in the degasser sump). Recycled flow rate was 1137 ± 80 L/min, and make-up water flow rate 24 ± 81 L/min, i.e., 97.9 ± 0.7% of the flow was recycled. Total system volume of RAS 1 during this period was 57.8 m³, and the daily system volume exchange rate thus 59.1 ± 19.5%, with a mean system HRT of 2 years. Then, at Day 20 after stocking, the fish (now 4.6 g/ind) were split from 9 into 15 tanks, the maximum dimensioned culture volume for RAS 1 (Table 3), and fed a total of 42 kg/day. Analyses of the feed sampled at four times during the 39 days long trial showed that it was composed of *as is*, average ± SD: 952 ± 4 g dry matter per kg feed, 502 ± 9 g crude protein per kg, 194 ± 2 g crude fat per kg, 110 ± 4 g ash per kg, and had an energy level of 21.8 ± 0.1 MJ per kg. The first water quality and treatment efficiency sampling was done at Day 21 (42 kg feed/day), and the second at Day 27 (49 kg/day). Due to a mistake, feeding was not done at Day 31, and to avoid sampling from a possibly unstable system, the next sampling was postponed until Day 39 (61 kg/day) (Table 4). Averaged for the whole testing period of Trial 2, the temperature was 13.4 ± 0.2 °C, pH 7.16 ± 0.01 (from online probes in the degasser sump), recycled flow rate was 1964 ± 15 L/min, and make-up water flow rate 11 ± 0 L/min, i.e., 99.4 ± 0.0% flow recirculation. Increment flow rate to 2200 L/min, as required from dimensioning (Table 3), was not attempted because it was found that the microscreen would likely not operate properly (i.e. it requires regular stops of the belt screen to accumulate a particle mat), above ~2000 L/min. Total system volume of RAS 1 during Trial 2 was 76.9 m³, resulting in a daily system volume exchange rate 20.6 ± 0.0%, i.e., a system hydraulic retention time of ~5 days.

As the total daily feed loading increased during Trial 2, so did the concentrations of several compounds, at most of the sampled locations in RAS 1, such as CO₂, TAN, nitrate, nitrite, and COD, while tank outlet pH decreased (Table 4). It is noteworthy, however, that neither the CO₂ nor TAN increased to concentrations above the water quality limits set for the maximal load situation (Table 2), before 134% of theoretical feed capacity was reached at Day 39, as shown in Fig. 8A. At this time, TAN in the tank outlet was 0.69 ± 0.05 mg/L (Table 4, Fig. 8A), i.e. similar to the limit for RAS 1 of 0.7 mg/L (Tables 2 and 3), and the outlet concentration for CO₂ was 9.1 mg/L compared to a limit of 10 mg/L (Table 4, Fig. 8A). In contrast, the system could not keep nitrite at the limit of 0.1 mg/L in the tank outlet, at any time during Trial 2, and averaged 0.22 mg/L NO₂-N. The reasons for these higher than designed nitrogen concentrations are unclear. However, sub maximal oxygen concentration in the bioreactor, which may lead to nitrite accumulation (Chen et al., 2006), is unlikely to explain the disagreement. The MBBR was heavily mixed by aeration and water left the culture tanks at a minimum of 8.6 mg/L O₂ (Table 4), from where the water was quickly returned to mechanical filtration and the MBBR. It is noteworthy however, that the nitrite concentration was stable throughout even at increased feed loading (Table 4), suggesting that the nitrite levels were not due to an incompletely matured biofilm leading to a transient spike. The chloride concentration was 111 mg/L at Day 39 (Hach Digital Titrator, method 8207), resulting in a Cl: NO₂-N ratio of 505:1, which should protect Atlantic salmon parr from adverse effects of nitrite, such as growth rate reductions and nitrite plasma accumulation (Svobodová et al., 2005; Gutierrez et al., 2011). Nitrite removal in MBBRs for aquaculture applications should be studied in more detail, to obtain better agreement between planned and actually measured nitrite levels.

The reason that the TAN and CO₂ limits used in dimensioning were not reached before feeding at 134% of theoretical capacity may be related to (1) the model used for fish TAN and CO₂ production (Timmons and Ebeling, 2007) overestimated the actual excreted TAN and CO₂, or (2) the RAS was over-dimensional in terms of TN and CO₂ removal capacity. In terms of the latter alternative, the TAN and CO₂ removal efficiencies were 84–89% and 57–67%, respectively (Table 4). In the case of TAN, these removal efficiencies are comparable to the required removal efficiency of 92% used in dimensioning (Table 3), but much higher than reported for MBBR systems previously (Rusten et al., 2006), and as high as achieved with fluidized-sand biofilters that are used in trout and salmon systems (Summerfelt, 2006). The MBBR in RAS 1 was designed to provide a mean TAN removal rate of 0.22 g TAN per day per square meter of media. However, at the three feed loadings tested in Trial 2, the actual areal nitrification rate ranged from 0.08 to 0.153 g TAN per day per square meter of media, as calculated from water flow rate times the change in TAN across the MBBR divided by the total media surface area. The areal nitrification rates reported here were...
Table 4
System parameters, water quality, and treatment efficiencies in Trial 2, during different daily feed loadings.

| Treatment unit | Parameter | Unit | 42 kg feed/day (93% of capacity)a | 49 kg feed/day (108% of capacity) | 61 kg feed/day (134% of capacity) |
|----------------|-----------|------|----------------------------------|----------------------------------|----------------------------------|
|                |           |      | Influent | Effluent | TE (%) | Influent | Effluent | TE (%) | Influent | Effluent | TE (%) |
| System         | Recycled flow | L/min | 1973 ± 4 | – | – | 1974 ± 4 | – | – | 1936 ± 3 | – | – |
|                | Make-up flow | L/min | 11 ± 0 | – | – | 11 ± 0 | – | – | 11 ± 0 | – | – |
|                | Recirculation | % | 99.4 ± 0.0 | – | – | 98.4 ± 0.0 | – | – | 98.4 ± 0.0 | – | – |
|                | Daily water excl. | % | 20.6 ± 0.0 | – | – | 20.6 ± 0.0 | – | – | 20.6 ± 0.0 | – | – |
| Culture tank   | Tank flow | L/min | – | – | – | – | – | – | 142 ± 0.9 | – | – |
|                | Ind. weight | g/ind | 4.56 | – | – | – | – | – | 9.37 ± 1.15 | – | – |
|                | Fish density | kg m⁻³ | 24 | – | – | – | – | – | 48.4 ± 7.4 | – | – |
|                | Temperature | °C | 12.9 ± 0.1 | 12.8 ± 0.0 | – | 13.6 ± 0.0 | 13.6 ± 0.0 | – | 14.3 ± 0.0 | 14.1 ± 0.0 | – |
|                | pH | 7.49 ± 0.01 | 7.14 ± 0.07 | – | 7.41 ± 0.01 | 7.02 ± 0.05 | – | 7.43 ± 0.02 | 6.75 ± 0.04 | – |
|                | Alkalinity | mg/L | 32 ± 4 | 28 ± 2 | – | 33 ± 4 | 33 ± 2 | – | 36 ± 0 | 39 ± 6 | – |
|                | Conductivity | uS cm⁻¹ | 580 ± 1 | – | – | 867 ± 3 | – | – | 1297 ± 2 | – | – |
|                | CO₂ (probe)c | mg/L | 1.0 ± 0.0 | 4.5 ± 0.9 | – | 2.0 ± 0.0 | 5.3 ± 1.2 | – | 2.2 ± 0.3 | 9.2 ± 1.0 | – |
|                | CO₂ (TIC)d | mg/L | 1.5 ± 0.1 | 3.2 ± 0.8 | – | 1.9 ± 0.1 | 5.0 ± 0.5 | – | 2.8 ± 0.5 | 10.8 ± 0.8 | – |
|                | TAN | mg/L | 0.03 ± 0.00 | 0.29 ± 0.10 | – | 0.04 ± 0.00 | 0.40 ± 0.04 | – | 0.10 ± 0.02 | 0.69 ± 0.05 | – |
|                | O₂e | % sat. | – | 89.9 ± 3.2 | – | 90.7 ± 0.4 | – | – | 86.7 ± 1.0 | – | – |
| Microscreen    | Temperature | °C | 12.8 | 12.8 | – | 13.7 | 13.6 | – | 14.3 | 14.1 | – |
|                | pH | 7.06 | 7.15 | – | 6.98 | 6.98 | – | 6.87 | 6.75 | – |
|                | Conductivity | uS cm⁻¹ | 576 | – | – | 867 | – | – | 1302 | – | – |
|                | TSS | mg/L | 3.1 | 1.3 | 58 | 2.5 | 0.8 | 68 | 4.0 | 2.9 | 28 |
|                | Turbidity | NTU | 0.80 | 0.81 | – | 0.39 | 0.45 | – | 0.91 | 0.80 | – |
|                | COD | mg/L | 14.9 | 14.9 | – | 17.7 | 17.5 | – | 24.5 | 24.1 | – |
| MBBR           | Temperature | °C | 12.8 | 12.8 | – | 13.7 | 13.6 | – | 14.3 | 14.1 | – |
|                | pH | 7.09 | 7.11 | – | 6.99 | 7.04 | – | 6.90 | 6.78 | – |
|                | Alkalinity | mg/L | 32 | 32 | – | 36 | 28 | – | 44 | 36 | – |
|                | Conductivity | uS cm⁻¹ | 582 | – | – | 870 | – | – | 1297 | – | – |
|                | TAN | mg/L | 0.31 | 0.03 | 89 | 0.37 | 0.04 | 89 | 0.62 | 0.10 | 84 |
|                | NO₂-N | mg/L | 0.22 | 0.25 | – | 0.22 | 0.22 | – | 0.08 | 0.19 | – |
|                | NO₃-N | mg/L | 26.1 | 26.4 | – | 51.1 | 46.2 | – | 85.3 | 86.2 | – |
|                | CO₂ (TIC) | mg/L | 4.1 | 3.6 | 14 | 5.8 | 4.6 | 21 | 8.4 | 7.7 | 8 |
|                | COD | mg/L | 15.8 | 13.2 | – | 17.5 | 17.4 | – | 23.4 | 28.0 | – |
| Degasser       | Temperature | °C | 12.8 | 12.7 | – | 13.7 | 13.6 | – | 14.3 | 14.1 | – |
|                | pH | 7.04 | 7.51 | – | 6.97 | 7.40 | – | 6.92 | 7.19 | – |
|                | Alkalinity | mg/L | 26 | 26 | – | 34 | 30 | – | 32 | 36 | – |
|                | Conductivity | uS cm⁻¹ | 580 | – | – | 870 | – | – | 1298 | – | – |
|                | CO₂ (probe) | mg/L | 4.3 | 1.5 | 67 | 4.7 | 2 | 57 | 8 | 3 | 63 |
|                | CO₂ (TIC) | mg/L | 4.3 | 1.3 | 69 | 4.9 | 2.4 | 50 | 6.5 | 3.5 | 47 |

a Percentage of theoretical capacity calculated from Tables 2 and 3; 45.4 kg/day is 100% of theoretical capacity.
b Apparent treatment efficiency, TE (%) = (C₁ – C₂) × 100/(C₁ C₉), where C₁ is concentration in the inlet to the treatment device, and C₉ is the outlet concentration. For the degasser, the outlet concentration refers to samples taken in the pump sump.
c Measured using an Oxysure CO₂ Portable analyser (Birkerød, Denmark).
d Calculated from TIC concentration, pH, and temperature, and using carbonate system constants in Summerfelt et al. (2001). At the 61 kg feed/day sampling point, CO₂ (TIC) concentration for only one tank is reported due to analytical difficulties.
e % O₂-saturation in the tank is the average ± SD of readings each fifth minute during the sampling periods (09:30–11:00 h).

similar to the areal rates for three types of MBBR media evaluated by Pfeffer and Wills (2011) at a low feed loading in 12 pt brackish water at 25 °C, but approximately half of the areal nitrification rate that Pfeffer and Wills (2011) reported at a high feed loading. The present areal nitrification rates were comparable to the rates reported by Rusten et al. (2006), but as shown in Fig. 8B, the measured removal rates were highest at the lowest effluent TAN concentrations, than predicted from Rusten et al. (2006). This high removal at low concentrations may have contributed slightly to the lower than expected TAN culture tank levels at the theoretical maximal feed capacity, since the MBBR effluent and tank inlet TAN (Table 4) was lower than predicted (Table 3) at the two first sampling points. Regarding CO₂, a removal efficiency of 78% was assumed in the dimensioning for the forced-ventilated cascade aeration column with 1.6 m packing depth, but only 57–67% (probe) or 47–69% (CO₂ calculated from TIC) was observed at an inlet CO₂ concentration of 4.3–8.0 mg/L (Table 4). However, it was observed that the MBBR also removed some CO₂ (Table 4), at an efficiency of 8–21%. As a consequence, the total system CO₂ removal efficiency was as expected 71–83% until 135% of theoretical feed capacity was reached, when it had declined to 55%.

The unit removal efficiencies are therefore unlikely to be the main reason for the observed deviation between theoretical and actual daily feed capacity, in terms of tank outlet TAN or CO₂ concentrations. However, the model by Timmons and Ebeling (2007) assumes a N-retention in the fish of 42%, which is low compared to published data using modern feeds in Atlantic salmon. In a study on 80 g initial weight Atlantic salmon parr (Grisdale-Helland and Helland, 1997), a N-retention of 52.8%, and a fecal N loss of 7.7% of feed N was found for parr fed the 51:25 protein:fat diet; a diet composition comparable to the diet used in Trial 2. Assuming a 50% leaching of fecal N to the rearing water (Smith et al., 1980), before the particles are removed in the microscreen, a total of 43.3% of ingested N would have been transferred to the water. In the case
of a 50% crude protein feed, as assumed in the dimensioning and used in Trial 2, this equals a loss of 34.6 g N per kg feed ingested, in contrast to the model (Timmons and Ebeling, 2007) used in dimensioning of RAS 1, which assumed a loss to the water of 46 g N per kg feed (Table 3). Using the loss of 34.6 kg N kg feed, the maximum daily TAN production assumed in the dimensioning (Table 3, 2083 g TAN/day), is reached at 60.4 kg feed/day, which equals Day 39 in Trial 2, when indeed water quality limits in TAN and CO₂ were met (Table 4). The general steps in dimensioning described in Summerfelt and Vinci (2004a,b) and Timmons and Ebeling (2007) appear sound, but it may be advisable to use published N-retention for the species and life-stage in question, when sizing biofilters. If the MBBR size had been reduced by 34%, investment for the entire NCRA water treatment would have been decreased by about 4%. Hence, in a research facility a higher TAN removal capacity may have a relatively minor influence on investment, but could provide increased experimental flexibility. Regarding the deviation for CO₂, an explanation may also lie in an overestimation of production, for instance due to efficient feed conversion with low O₂ consumed and CO₂ produced by the fish.

Although Trial 2 was not designed as a growth study, it was found that the average growth rate from Day 21 to Day 39 was 4.2% day⁻¹, which is similar to that assumed for the maximal load situation (Table 2), as well as being similar to growth rates observed in the industry (Skretting, 2007, 4.2%/day for 1–5 g/in.d, and 4.0% for 5–15 g/in.d, at 14°C). While Trial 1 was run at a relatively light feed load and TAN and NO₃-N, and presumably other compounds, were at low concentrations, Trial 2 indicates that the RASs in NCRA could support rapid growth also at a maximal load situation.

4. Conclusions

The facility described here was built for scientific purposes, and for focus on the environmental requirements of Atlantic salmon in RAS. A major goal when constructing the facility was to offer sufficient flexibility for experiments, and for doing such experiments on a semi-commercial scale. During the 3 years of operations so far, the facility has been in constant use and five publicly funded experiments, and four industry-funded projects, have been completed. Trials 1 and 2 indicated that the facility could maintain the relevant growth rates of Atlantic salmon, and that the water quality limits, except NO₃-N, were met at the intended daily feed load. However, it is advised that dimensioning of TAN removal in the future employ published N-retention data, when available, for the species and life-stage in question. In the years to come, it is expected that NCRA will be useful in providing knowledge about fish requirements in RAS.

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