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# Startup and effects of relative water renewal rate on water quality and growth of rainbow trout (*Oncorhynchus mykiss*) in a unique RAS research platform

Jani T. Pulkkinen[a,*], Tapio Kiuru[b], Sanni L. Aalto[c,d], Juha Koskela[a], Jouni Vielma[a]

[a] Natural Resources Institute Finland, Survontie 9A, 40500 Jyväskylä, Finland
[b] Natural Resources Institute Finland, Vilppulantie 415, 41340 Laukaa, Finland
[c] Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland
[d] Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

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ABSTRACT

The aquaculture industry is growing fast but facing two major challenges: a shortage of suitable locations for growth and the need to reduce environmental impacts. One solution for both these challenges is inland production through recirculating aquaculture systems (RAS). The RAS technique is rather new, and several practical issues need to be solved. In this study, an experimental platform, consisting of ten individual RAS units, was built for small-scale testing of different RAS designs and operation methods, and two preliminary experiments were conducted. In the first experiment, the capability of different chemical additions (sodium nitrite, ammonium chloride and/or cane sugar) to fasten the startup of the nitrification bioreactor was tested. In addition, the suitability and reliability of an online water measurement system in monitoring nitrification process with was evaluated. We demonstrated that when using a combination of sodium nitrite and ammonium chloride in a concentration of 5 mg $l^{-1}$, nitrification started one week before than when using only ammonium chloride or a clean start with rainbow trout (*Oncorhynchus mykiss*). In the second experiment, the effect of different relative water renewal rates (RWR) on water quality, rainbow trout growth and feed conversion ratio (FCR) were examined at 16 °C. Based on the results, FCR increased when RWR went below 478 l $kg^{-1}$, and the specific growth rate decreased when RWR went below 514 l $kg^{-1}$. Furthermore, when RWR decreased, nitrate, nitrite and organic material accumulated in the circulating water. In conclusion, we showed using experimental RAS platform that online water quality monitoring is a useful tool in following the effect of different management practices. Furthermore, we demonstrated that chemical substrate additions provide the fastest biofilter startup, and that water management is still in the key role in defining the fish production in RAS.

## 1. Introduction

The Finnish aquaculture sector has been in decline since the beginning of the 1990 s (Official Statistics of Finland, 2017), mainly because of the strict environmental regulations concerning nutrient discharges. To meet the strict wastewater regulations regarding phosphorus and nitrogen, novel methods for increasing aquaculture production and value are needed. Recirculating aquaculture systems (RAS) uses, and thus discharges, less water, which can be treated efficiently and economically compared to traditional flow-through and net cage aquaculture systems (Martins et al., 2010).

Although the principles of RAS techniques are a few decades old (Bohl, 1977; Rogers and Klemetson, 1985), the technique has shown clear signs of expanding to a commercial scale during the last decade (Sturrock et al., 2008; Bergheim et al., 2009; Dalsgaard et al., 2013a). In Finland, recirculating systems have been used in aquaculture for more than 10 years, but companies have had economic difficulties mostly due to technical problems. However, the two largest RAS farms have a projected capacity at total of some 4500 tons, and thus RAS is developing to be a potential technology alongside more traditional forms of aquaculture.

The various challenges of RAS technology have been well covered in the recent scientific literature (Badiola et al., 2012; Klinger and Naylor, 2012) and in the symposiums (e.g. in NORDIC RAS workshops: Dalsgaard et al., 2013b, 2015/, 2017). One central problem is the difficulty to manage solids production and biofilter function to maintain a

* Corresponding author.
*E-mail address:* jani.pulkkinen@luke.fi (J.T. Pulkkinen).

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sufficiently high water quality in RAS (Badiola et al., 2012). Another important issue to be better understood is the RAS microbiology. For example, the interactions between beneficial and harmful bacteria are still poorly known (Rurangwa and Verdegem, 2015). To meet these challenges in the RAS sector, experimental scale units, where different operational designs can be scientifically tested, are needed. In this study, the capacity and various functions of the experimental platform with were designed in a project group consisting of Finnish RAS farmers, technology suppliers and academia. The objective was to include similar water treatment units in the experimental system as used in typical modern RAS farms. Special attention was paid to the ability to adjust water treatment for different trials, online water quality measurements and accurate make-up water flow adjustment.

This study gives an overview of the research platform, its design and system startup. The functioning of the system and online water monitoring system were tested in two experiments. The aim of the first experiment was to examine the startup of nitrification by using chemical additions, and the determination of nitrification by the online monitoring system. The startup phase of the biofilter is considered to be long, e.g. 40 days in shrimp RAS (Perfettini and Bianchi, 1990), and it can be shortened by using inoculates of nitrification bacteria (Grommen et al., 2002; Kuhn et al., 2010). However, external bacteria load might be harmful, and the community composition of the bacterial inoculates might differ from the one of the natural RAS bacterial population, thus an alternative strategies for enhancing the startup of the nitrification process are needed. Here, we tested how the addition of high concentrations of nitrification substrates (ammonia and/or nitrite) will affect biofilter startup. In addition, we tested whether organic carbon addition in the startup phase was beneficial to microbial communities, since it could facilitate the biofilter adaptation to the high organic carbon loading released from feed and faeces after the fish introduction. In the second experiment, the aim was to investigate the effect of relative water renewal rates (RWR) on water quality, fish growth and feed-conversion ratio. The intensity of RAS is often described by how much fresh water is being introduced per feed used, which in turn will influence nitrate and solid accumulation in the system (Timmons and Ebeling, 2013). We hypothesized that under lowest water renewal rates, harmful substances such as nitrate and organic load would have negative effects on fish performance. Furthermore, these two experiments provided information about the operation of the water treatment and online water quality measurement system under different operational options.

## 2. Material and methods

### 2.1. RAS design

The RAS platform was built by Arvo-Tec Oy (Joroinen, Finland) in the existing research hall at the Laukaa fish farm of Natural Resources Institute Finland (Luke) (Fig. 1). The system consists of 10 individual units, each having an individual water treatment system and water quality monitoring system. In Table 1, the dimensioning of the water treatment units to remove TAN and $CO_2$ is provided.

Each unit consists of a bottom-drained rearing tank, solid removal systems, biological filtration systems and an aeration system. Each water treatment step can be by-passed and, therefore, the experimental setup can be varied. Each unit consists of (in water circulating order): a 500 l square plastic rearing tank (ArvoTec, Joroinen, Finland), feed collector unit, 24 cm diameter (hydraulic loading 133-531 l $min^{-1}$ $m^{-2}$) swirl separator (Eco-Trap Collector1, Pentair Aquatic Eco-Systems, Minneapolis, USA), drum filter with 60 µm filter panels (Hydrotech HDF501, Veolia, Paris, France), four separate 147 l bioreactor tanks, 0.8 m high trickling filter and 70 l pump sump (Fig. 2).

Water is pumped from the pump sump with a recirculating pump (Magna 3, Grundfos, Bjerringbro, Denmark) to the bottom-drained fish tank. Recirculating water pH is adjusted by single channel control unit (Dulcometer, ProMinent, Heidelberg, Germany) using pH-probe located in the pump sump and low-pressure metering pump (Beta b, ProMinent, Heidelberg, Germany) dosing diluted sodium hydroxide to the trickling filter prior to the pump sump.

Every fish tank has a galvanic oxygen probe (OxyGuard, Farum, Denmark) which is used with the monitoring system (Atlantic, OxyGuard, Farum, Denmark) to control the emergency oxygen diffuser located at the bottom of the tank. Emergency oxygen turns on in power failure situations and when oxygen saturation is below the adjusted set point level. Pure oxygen is added to the pump sump via ceramic diffusers to supersaturate the tank inlet water. Oxygen saturation in fish tanks is manually adjusted with constant flow regulator (Model 2851, Kytola® instruments Oy, Muurame, Finland).

The trickling filter is a forced-ventilated cascade aeration column designed to remove $CO_2$ from the circulating water. Filters are filled with Bio-Blok® 200 filter medium (EXPO-NET Danmark A/S, Hjørring, Denmark). Each trickling filter has a channel blower (Onnline CK 100 A, Onninen, Vantaa, Finland) on top of the filter, and the blower can be activated using a carbon dioxide set-point value. The blower is also connected to a seven-point thyristor controller for adjusting blowing speed.

Fish are fed with a commercial feeding system (T Drum 2000, Arvo-Tec, Joroinen, Finland). The tank light system is located in the tank cover. The cover is made from a special machined acrylic panel, which reflects LED light cast from the side of the panel (Aicci, Muurame, Finland). Light intensity and photoperiod can be adjusted.

Make-up water is pumped from a 600 l plastic tank with a water dosing pump (DDI-222, Grundfos, Bjerringbro, Denmark). In the RAS units, make-up water is added to the feed collector units. Make-up water alkalinity can be increased by adding sodium bicarbonate to the make-up water storage tank with an automated belt feeder. Inlet water is disinfected with UV light (Duv 01 A, Lit, Moscow, Russia) and it can be filtered with a string-wound cartridge (housing: Shelco RHS, Charlotte, USA, cartridge: MS10FP3, Shelco, Charlotte, USA) and carbon block filter (5FOS, Shelco, Charlotte, USA).

Laukaa fish farm's water source is lake Peurunkajärvi (62.44886, 25.85201), located 8 m above the farm. Farm uses water from two water depths (10 m hypolimnion and 4 m surface water) (Table 2). Water temperature can be adjusted by mixing the two inlet water sources. Water can be also heated or cooled with heat-exchangers and a heat-pump (30 HM-065, Carrier, Farmington, USA). In RAS units, water temperature is adjusted by controlling the air temperature by air conditioning system.

### 2.2. Data collection

Each RAS unit has a separate online water quality measurement system. Each fish tank has a spectrometer probe (spectro::lyser, s::can, Vienna, Austria), carbon dioxide sensor (Franatech, Lüneburg, Germany), pH probe (pH::lyser, s::can, Vienna, Austria) and optical oxygen probe (oxi::lyser, s::can, Vienna, Austria). The spectrometer probe measures water nitrate, nitrite, turbidity, total suspended solids, total organic carbon and UV 254 absorbance. Recirculating water flow is measured with a flow sensor (type 8012, Bürkert, Ingelfingen, Germany) located in the tank inlet water pipe. All data is collected at industrial on-line computer (con::cube, s::can, Vienna, Austria), which can be adjusted to take measurements at desired intervals. Computers can be accessed remotely over a VPN.

Drum filters are connected to a digital time counter (H7ET, Omron, Osaka, Japan), which calculates the operating time of the backwash. Time counter data is registered manually. Backwash water intake is located in the pump sump.

### 2.3. Nitrification bioreactor startup

Effects of different chemical additions for enhancing biofiltration